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RECENT ADVANCES
in
PHARMACOLOGY

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By

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With 46 Illustrations

CENTRAL FOOD TECHNOLOGICAL RESEARCH INSTITUTE
4 MAR 1951
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LONDON
J. & A. CHURCHILL LTD.
104 GLOUCESTER PLACE, W.1
1950

1400

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Recent advances

Printed in Great Britain

To the memory of A. J. Clark

A great pharmacologist and a lovable man

PREFACE

Recent discoveries of new drugs and advances in our knowledge of their actions and uses have been so numerous and widespread that even the specialist has great difficulty in keeping pace with the course of events. Our purpose in writing this book has been to survey some important recent developments in Pharmacology likely to be of interest to students and practitioners of medicine, and to present the material in such a way as to provide not only an account of our knowledge concerning the actions of drugs, but also a basis for their use in practice.

Modern developments in pharmacology have followed two main directions; the first, in the investigation of mechanisms by which drugs produce their actions, a field to which A. J. Clark made outstanding contributions, and the second, in the discovery of many drugs of therapeutic importance. The first development has been of value from a theoretical point of view, as a means of discovery of new drugs, and also in the more adequate application of drugs in practice. In the present book we have tried to describe these various developments, giving due emphasis to all branches of the subject. Thus we have dealt with many of the recently introduced techniques which have been used to elucidate the actions of drugs, and we have not only described the pharmacological actions and fate of drugs in the body, but have tried to emphasize the importance of this knowledge in their therapeutic use.

At the outset we realized that it would be quite impossible to review the whole vast and growing field of Pharmacology, and we have had to confine our discussion to what we considered at that time to be the most important aspects of the subject. We are fully aware that important material has been omitted; we have, for example, said little about the sulphonamides as they are no longer in the forefront of development, and our account of cortisone and adrenocorticotrophic hormone must necessarily be inadequate, since here advancing knowledge actually overtook us in the middle of our task.

The review of each individual topic is by no means exhaustive, but we have aimed to provide a sufficient number of important references to satisfy the reader who wishes to consult the original sources of information, and to facilitate matters we have marked review articles with an asterisk.

The question of drug nomenclature is a vexing one that offers no easy solution, but we have wherever possible used either the names given in the British Pharmacopoeia or those officially approved by the General Medical Council.

We should like to offer our thanks to those authors who have so kindly allowed us to reproduce figures and photographs (cited in the appropriate legend) and also to the Editors of *American Journal of Medical Sciences*, *American Journal of Psychiatry*, *Annals of Internal Medicine*, *Annals of Tropical Medicine and Parasitology*, *Annals of the New York Academy of Sciences*, *Archives of Internal Medicine*, *Blood*, *British Medical Journal*, *Bulletin of the Johns Hopkins Hospital*, *Endocrinology*, *Journal of Allergy*, *Journal of the American Medical Association*, *Journal of Biological Chemistry*, *Journal of Clinical Investigation*, *Journal of Clinical Pathology*, *Journal of Immunology*, *Journal of Neurophysiology*, *Journal of Pharmacology and Experimental Therapeutics*, *Lancet*, *Proceedings of Staff Meetings of the Mayo Clinic*, *Quarterly Journal of Medicine*, and to The Year Book Publishers, Inc., Chicago, and The Williams and Wilkins Company, Baltimore.

We should like to say how grateful we are to numerous colleagues at Guy's Hospital and Middlesex Hospital Medical Schools for helpful discussions of the various subjects dealt with in this book and we wish to acknowledge in particular the valuable assistance we have received from Professor W. A. Bain, Professor A. S. V. Burgen, Mr. W. F. Floyd, Dr. T. A. B. Harris, Professor G. A. D. Haslewood, Dr. A. J. H. Hewer, Dr. Bernard Johnson, Dr. J. A. Nissim, Colonel H. E. Shortt, and Professor R. H. S. Thompson.

We are also greatly indebted to Miss B. Senior, Miss G. Murgatroyd and Miss P. Howes for their helpful co-operation in preparing the manuscript and illustrations.

And finally we should like to express our deep appreciation of the constant and untiring help which Mrs. S. Robson has given us in the writing of this book.

We also wish to express our appreciation of the most cordial help which we have consistently received from Messrs J. & A. Churchill Ltd.

J. M. Robson
C. A. Keele

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CHAPTER 1

ANTI-CHOLINESTERASE DRUGS

In this chapter the actions of anti-cholinesterase drugs, with particular reference to the newly discovered alkyl phosphates, are discussed. A brief account of chemical transmission precedes a discussion of the mechanisms of cholinesterase inhibition. The actions of these drugs on neuromuscular transmission are described in some detail and will be further referred to in Chap. 2 in discussing the mode of action of curare. The central nervous actions of these drugs are only briefly mentioned. For this and other aspects of anti-cholinesterase drug action the reader is referred to the excellent review on anti-cholinesterase drugs by Koelle and Gilman (1949).

Chemical Transmission

The work of Loewi, and of Dale and his colleagues, has established the importance of chemical agents as transmitters of activity in the autonomic nervous system, at the junction of motor nerve and skeletal muscle, and probably also in the central nervous system. For example, at the preganglionic nerve endings of the autonomic nervous system, acetylcholine (A.Ch.) is liberated by nerve impulses in both sympathetic and parasympathetic divisions. The A.Ch. so released then stimulates the ganglion cells, from which arise nerve impulses which pass along the postganglionic nerve fibres and in turn liberate transmitter agents from their nerve endings; postganglionic parasympathetic nerve fibres produce and liberate A.Ch. whereas most of the postganglionic sympathetic fibres produce and liberate adrenaline (and probably nor-adrenaline, e.g. von Euler, 1946, 1948). These substances then act upon the structures innervated by this system.

In the resting state the chemical transmitters are apparently held in the nerve fibre endings in a physiologically inactive form. The passage of a nerve impulse is required for the liberation of the transmitter in a physiologically effective state. Dale has suggested that nerve fibres which form and liberate A.Ch. should

be termed "cholinergic", and that those which form and liberate adrenaline should be called "adrenergic."

The factors to be considered in relation to the action of chemical transmitters will now be illustrated by a discussion of *Neuromuscular Transmission*.

The motor nerve fibres to skeletal muscle were shown by Dale, Feldberg and Vogt (1936) to be cholinergic in character, since motor nerve stimulation caused the appearance of A.Ch. in the fluid collected from the perfused tongue of the cat. They calculated that each motor nerve impulse liberated 0.00002 μg. of A.Ch. It was further shown by Brown, Dale and Feldberg (1936) that intra-arterial injection of A.Ch. (e.g. 2 μg.) close to the gastrocnemius muscle of the cat could produce a contraction equal in magnitude, though slower in time course, to that which followed a motor nerve volley. Buchthal and Lindhard (1942) applied minute amounts of A.Ch. directly to the end-plate region in lizard muscle and observed a propagated contraction of the muscle fibre, which was not produced by even higher concentrations of A.Ch. in contact with other parts of the fibre.

The acceptance of A.Ch. as the neuromuscular transmitter must also depend on the demonstration of mechanisms for its rapid removal or inactivation. It is generally thought that the enzyme *cholinesterase* plays the most important rôle in the inactivation of liberated A.Ch., but other factors such as resynthesis by the nerve terminals from which it was released, and diffusion into the surrounding medium, might also be of some significance. Cholinesterase hydrolyses A.Ch. to acetic acid and choline, and thereby virtually annihilates its powerful physiological actions. In skeletal muscles Marnay and Nachmansohn (1938) calculated that there were sufficiently high concentrations of cholinesterase at the motor end-plate region to account for the inactivation of A.Ch. within the refractory period of the muscle. Burgen (1949) has also suggested that the liberated A.Ch. is rendered physiologically inactive as soon as it becomes fixed by cholinesterase, before any hydrolysis takes place. There is therefore little difficulty in accounting for the very brief duration of action of the A.Ch. released by a single motor nerve volley.

The physiological actions of A.Ch. on muscle are probably due to its combination with specific receptors on the surface of the motor end-plate. The studies of Eccles and O'Connor (1939),

Eccles, Katz and Kuffler (1941) and Eccles (1948) have demonstrated the existence of a negative electrical potential change at the end-plate region ("end-plate potential," e.p.p.) immediately following the arrival of an impulse at the motor nerve endings, and just preceding the "spike potential" associated with propagated muscular contraction. The work of Kuffler (1943) shows that the e.p.p. represents a depolarization of the end-plate membrane, and that the depolarizing agent is A.Ch. When the e.p.p. exceeds a certain critical value a spike potential is set up, and propagated muscular contraction occurs.

The Action of Drugs on Neuromuscular Transmission

The description of the sequence of events which follows the release of A.Ch. from motor nerve endings reveals possible ways in which drugs might modify the normal transmission process.

(1) Acetylcholine liberation might be increased or decreased. It is doubtful whether any substances directly *increase* the quantity of A.Ch. released at motor nerve endings, but it is highly probable that A.Ch. liberation is *decreased* by procaine, which acts by blocking conduction in the motor nerve fibres or their endings (Harvey, 1939; Jaco and Wood, 1944). This action would account in part for the "curare-like" effect of this drug. It has recently been shown by Burgen *et al.* (1948) that Botulinum Toxin also causes paralysis by diminishing A.Ch. liberation at motor nerve terminals. This effect differs, however, from that of procaine in two ways. Firstly, the toxin develops its action very slowly, even when applied in high concentrations directly to a nerve-muscle preparation; this is probably due to the large size of the toxin molecule, which is a protein with a molecular weight of about 1,000,000. Secondly, the action of this toxin is irreversible.

(2) The activity of liberated A.Ch. might be modified.

(*a*) A substance might react chemically with A.Ch. before its arrival at the end-plate or cholinesterase receptors. No such action is known.

(*b*) The physiological activity of released A.Ch. could be *enhanced* by any drugs which reduce its rate of inactivation. The anti-cholinesterase drugs, e.g. *Physostigmine* and *Neostigmine*, act in this way, by combining with the enzyme cholinesterase. They apparently become attached to the same enzyme receptors by

which A.Ch. is normally fixed prior to hydrolysis. By thus preventing the attachment of A.Ch. to cholinesterase, physostigmine allows the normally liberated physiologically active A.Ch. to rise to abnormally high concentrations. Acting in this way, physostigmine potentiates the muscular response to motor nerve stimulation or injected A.Ch. (Brown *et al.*, 1937).

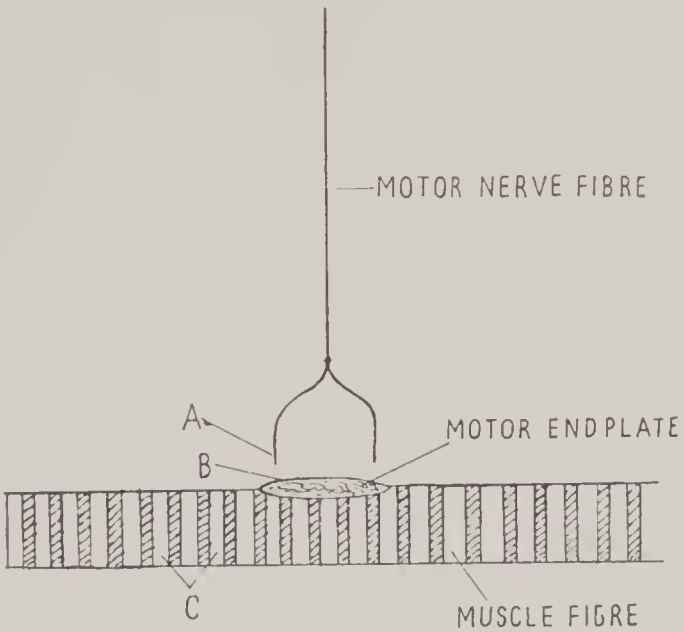


FIG. 1

DIAGRAM OF NEUROMUSCULAR JUNCTIONAL REGION

Procaine and Botulinum Toxin block conduction at the nerve ending (A). *Acetylcholine* stimulates the motor end plate (B). *Tubocurarine* also acts at B to block the action of A.Ch. *Potassium* (partly) and *Adrenaline* stimulate the muscle fibre directly (C).

(*c*) The physiological activity of released A.Ch. could be reduced by drugs which prevent its combination with the specific motor end-plate receptors. This appears to be the mode of action of curare in the production of skeletal muscular paralysis. It was shown by Dale *et al.* (1936) that curare did not reduce A.Ch. liberation from motor nerve endings even when the muscle was completely paralysed. This mode of action is comparable to that by which atropine prevents the action of A.Ch. on visceral struc-

tures, e.g. heart, intestine and glands. It is, however, important to note that atropine, in the usual dose range, does not block the action of A.Ch. on skeletal muscle, nor does curare prevent the visceral effects of A.Ch. These facts may be summarized by saying that atropine antagonizes the "muscarine" actions, and curare the "nicotine" actions of A.Ch.

(3) The response of the muscle fibre itself might be directly affected by drugs. Brown (1937) attributed some of the potentiating action of potassium to such a mechanism, and a recent analysis of the mode of action of adrenaline on skeletal muscle shows that it probably acts directly to increase the tension developed in the individual fibre (Brown, Bülbring and Burns, 1948).

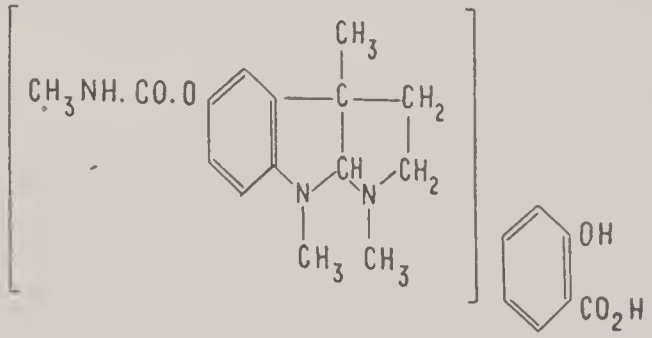
Anti-cholinesterase Drugs

Many substances have been described as antagonists of cholinesterase (as shown by *in vitro* studies) but it is proposed here to deal only with the really powerful cholinesterase inhibitors (and, more particularly, the recently discovered ones) whose *in vitro* activity can be demonstrated in concentrations of 10^{-8} Molar, or less. The important members of this group are :—

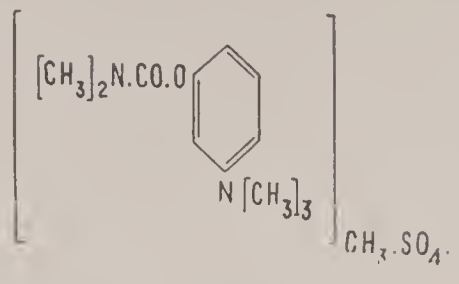
(i) *Physostigmine*, an alkaloid obtained from *Physostigma venenosum* (Calabar bean)
(ii) *Neostigmine* (Prostigmine)
(iii) *Diisopropylfluorophosphonate* (DFP)
(iv) *Hexaethyltetraphosphate* (HETP)
(v) *Tetraethylpyrophosphate* (TEPP)

The formulæ of these compounds are shown below.

Chemical Formulæ of Anti-cholinesterase Drugs

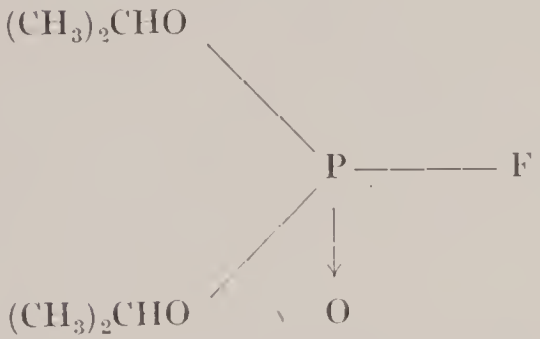


Physostigmine Salicylate



Neostigmine Methylsulphate

Dimethylcarbamic ester of 3-hydroxyphenyltrimethyl-ammonium methyl sulphate



Diisopropylfluorophosphonate (DFP)

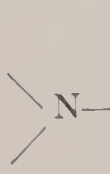
Tetraethylpyrophosphate (TEPP)

The constitution of hexaethyltetraphosphate (HETP) is still uncertain. It consists of a mixture of phosphorus esters of which TEPP is the main active constituent

Mode of Action

These drugs all produce pharmacological actions similar to those of A.Ch., and since they inhibit the destruction of the latter by cholinesterase *in vitro* it is natural to ascribe their actions

in vivo to this property. The preservation of A.Ch. by physostigmine in perfused animal tissues was an essential part of the work which established the importance of A.Ch. as a chemical transmitter, and it has been shown that the other drugs mentioned can also inhibit the *in vivo* destruction of A.Ch. The only question still at issue is whether these substances also produce effects by actions independent of their anti-cholinesterase properties.

The chemical structure necessary for anti-cholinesterase activity is not clear. It was originally suggested by Stedman and Stedman (1931) that the presence of the urethane group  , as found in physostigmine and neostigmine, was essential, but the powerful inhibition of cholinesterase by DFP, and the alkylpolyphosphates, HETP and TEPP, which contain no urethane group, shows that other possibilities must be considered.

Types of Cholinesterases

The correlation of *in vitro* inhibition of cholinesterase with the *in vivo* pharmacological actions of anti-cholinesterase drugs has recently become more complicated with the discovery of at least two types of cholinesterase in body fluids and tissues. Mendel and Rudney (1943) have shown the existence of two enzymes, "True" and "Pseudo"-cholinesterase [which appear to correspond to "acetylcholinesterase" and "cholinesterase" described by Nachmansohn and Augustinsson (1948)]. True cholinesterase acts best on low concentrations of A.Ch., and also readily hydrolyses acetyl-β-methylcholine, but not benzoylcholine, whereas pseudo-cholinesterase acts best on high concentrations of A.Ch. and readily hydrolyses benzoylcholine, but not acetyl-β-methylcholine. The distribution of these two varieties of cholinesterase is important in relation to the mode of action of anti-cholinesterase drugs. The pseudo-form is found mainly in plasma and is probably of little physiological importance ; the true form occurs in erythrocytes, brain, muscle and other tissues and is obviously adapted to deal with the A.Ch. released at cholinergic nerve endings. By using acetyl-β-methylcholine and benzoylcholine as substrates, *in vitro* studies can indicate the relative

proportions of the two cholinesterases in the tissue under investigation.

In assessing the mode of action of anti-cholinesterase drugs the possibility of differential inhibition of the two forms of cholinesterase must be considered. It has been found, for example, by Hawkins and Mendel (1947) that DFP is 100 times more powerful an inhibitor of pseudo- than of true cholinesterase, so it is not surprising that Mazur and Bodansky (1946) observed no symptoms in man even when DFP had produced 99 per cent inhibition of plasma cholinesterase activity. Hawkins and Mendel (1947) noted symptoms in rabbits only when the true plasma esterase activity was depressed to about 25 per cent of its original level, and similar values for brain cholinesterase inhibition in relation to symptoms in rats were recorded by Koelle and Gilman (1946). Riker and Wescoe (1949) have studied quantitatively the relation between cholinesterase inhibition and physiological response in the submaxillary gland of cats. After administration of DFP, up to 50 per cent of the glandular cholinesterase could be inhibited without affecting the response to nerve stimulation. With more than 50 per cent inhibition there were signs of enhanced secretory activity in response to nerve stimulation, and when 90 per cent of the glandular cholinesterase was inactivated spontaneous salivary secretion set in. It would be interesting so to correlate increasing degrees of cholinesterase inhibition with functional activity in other organs. When DFP is injected into the body, it will combine firstly with pseudo-cholinesterase in the blood and tissues, and only when this is "saturated" will it react significantly with true cholinesterase and lead to A.Ch. accumulation at cholinergic nerve endings. Burgen (1948) has shown that *in vitro*, physostigmine, neostigmine and TEPP also combine more readily with pseudo- than with true cholinesterase, but the differences in affinity are less than with DFP.

Duration of Anti-cholinesterase Drug Action

Burgen (1949) has described *in vitro* studies of the kinetics of cholinesterase inhibition which explain the differenecs in duration of action of the various anti-cholinesterase drugs. Physostigmine and neostigmine show typically what is described as competitive reversible inhibition of cholinesterase ; these drugs are thus readily removed from their combination with cholines-

terase, leaving the enzyme unchanged, so their duration of action is brief. On the other hand DFP and TEPP appear to react with cholinesterase in two stages :—

(1) A reversible competitive combination which precedes

(2) An irreversible inactivation of the enzyme, which is responsible for the prolonged duration of action of these compounds. Recovery of cholinesterase activity in this case depends on synthesis of new enzyme. The initial combination between DFP (or TEPP) and cholinesterase is inhibited by the presence of physostigmine or neostigmine. This antagonism has also been shown to occur *in vivo* (Koster 1946 ; Hobbiger, 1949) and will be discussed further in the account of myasthenia gravis. DFP is even longer acting than TEPP ; this is related to differences in properties between the diisopropyl and diethylphosphoryl radicals (Burgen 1949).

These *in vitro* studies of the actions of anti-cholinesterase drugs would seem to support strongly the view that their pharmacological actions *in vivo* are due to inhibition of cholinesterase. Certain differences between members of the group may be due to differences in distribution in the body, which in turn may be related to differences in physical properties, e.g. lipoid solubility. There are, however, some facts which suggest that anti-cholinesterase drugs may act independently of their esterase inhibiting properties. For instance, Riker and Wescoe (1946) observed a contracture in the denervated cat gastrocnemius following intra-arterial injection of 25μg. of neostigmine, after 40 times this dose of DFP had produced no effect. This suggests that neostigmine may have a direct stimulating action on skeletal muscle.

DFP

General Account of the Actions of DFP, HETP and TEPP

Diisopropylfluorophosphonate was first prepared in 1942 by McCombie and Saunders after it had been shown by Adrian, Feldberg, Kilby and Kilby (1941) that dimethylfluorophosphonate was a cholinesterase inhibitor, and that its pharmacological actions resembled those of other anti-cholinesterase drugs such as physostigmine. Exposure to the vapours of these fluorophosphonates in man produced a long-lasting miosis, ciliary spasm, and respiratory difficulty attributable to bronchiolar muscle spasm (Adrian *et al.*, 1947).

Studies of the actions of DFP in animals have revealed the muscarinic and nicotinic types of effect seen with physostigmine. In dogs and cats, for example, the *muscarinic* actions include profuse salivation, increased secretion in the glands of the respiratory tract, bronchospasm, and hyperperistalsis leading to defecation; the *nicotinic* effects include muscular twitching and tremors, which may be followed by convulsions due to stimulation of the central nervous system.

Heymans and Jacob (1947) noted an important difference between DFP and physostigmine in the anæsthetized dog. Physostigmine was shown to potentiate the bradycardia which follows vagal stimulation whereas DFP had no such action, even when the cholinesterase activity of heart muscle had been almost abolished.

DFP on Neuromuscular Transmission

I. Actions in the Cat. The actions of DFP on *neuromuscular transmission* have been studied by Brown, Burns and Feldberg (1948). They observed that DFP produced much less muscular twitching and fibrillation than physostigmine. In decerebrate cats close arterial injection of 200 μg. of DFP produced an increase in the tension developed by the tibialis anterior muscle (stimulated maximally through its motor nerve at 10 sec. intervals), comparable to that produced by arterial injection of 20 μg. of physostigmine (Bacq and Brown, 1937). The actions of DFP are, however, qualitatively very similar to those of physostigmine; the repetitive contraction following a single motor nerve volley (which is responsible for the potentiation), the production of a poorly sustained contraction with high frequency stimulation, and the depressed response to single nerve volleys after arterial injections of A.Ch., are seen with both drugs. Physostigmine and DFP are about equally active against true cholinesterase (Burgen, 1948). It was found by Hunt (1947) that DFP was less effective than neostigmine as an antagonist to curare.

II. Actions in Normal Man. The actions of A.Ch., neostigmine and DFP on neuromuscular transmission in *normal man* have been fully studied by Harvey and his colleagues (1941, 1946).

(1) Rapid injection of A.Ch. (10–50 mg.) into the brachial artery produced flushing and profuse sweating in the area of distribution of this artery. Excruciating pain lasting about 10 sec.

was felt in the hand and forearm ; at the same time, but outlasting the pain by 10–20 sec., muscular power was greatly reduced, with complete recovery in 30–60 sec. This would seem to be an example of the well-known depressant effect of high concentrations of A.Ch. Brief localized twitches in palms and forearm muscles were also seen.

(2) *Neostigmine.* Intra-arterial injection of 0.2–1.5 mg. of neostigmine (after 1–2 mg. of atropine) produced slight flushing of the skin, very little sweating and no pain. There was, however, profound weakness of finger, hand, and wrist movements, so that for 1–3 minutes the subject could not grip the dynamometer. Power began to return in 5 minutes and was normal in 20–45 minutes. Muscular fasciculations (i.e. spontaneous, small, irregular twitchings) appeared within 15 sec. of the injection and lasted 10–30 minutes.

The electromyogram of the hypothenar muscles was recorded in these experiments by means of a surface electrode, and the ulnar nerve was stimulated supramaximally at the elbow. In the normal state a single motor nerve volley produced a single diphasic potential response of constant size. After neostigmine a single nerve volley produced a repetitive discharge, consisting of 3 or 4 potentials of progressively diminishing magnitude. When two nerve volleys were given close together (e.g. 10–100 m.sec. apart) the second potential response was equal to the first in the normal condition, but was markedly reduced in size after neostigmine. These findings agree with the electrical studies of Brown (1937) on the action of physostigmine, and support the hypothesis of A.Ch. transmission at motor nerve endings.

(3) *DFP.* Harvey *et al.* (1946) have investigated the effects of intra-arterial DFP on the normal human electromyogram. 0.5–2 mg. of DFP produced sweating, a sensation of coldness in arm and hand, and fasciculations in the muscles of the injected area (which lasted for about nine days). Motor weakness was marked and persisted for long periods, e.g. for up to eleven weeks. The electromyographic responses to single motor nerve volleys, and to two close nerve volleys, were similar to those seen after neostigmine. This extremely prolonged action of DFP may be related to the irreversible character of its combination with cholinesterase, recovery of normal activity depending on resynthesis of this enzyme. The reaction of neostigmine with

cholinesterase is easily reversible and its duration of action correspondingly brief.

The actions of A.Ch., neostigmine and DFP in myasthenia gravis will be discussed later.

DFP on the Central Nervous System

The production of convulsions with large doses of DFP in animals has been described. A detailed investigation of the effects of intravenous and intrathecal administration of DFP on the knee jerk, and extensor and flexor reflex of the cat, has been reported by Chennells and Wright (1947). They demonstrated a central stimulant action very similar to that of physostigmine. It was suggested by Schweitzer *et al.* (1939) that the central nervous stimulant actions of physostigmine were related to its being a tertiary amine, which in the free base form is lipoid soluble and can therefore penetrate into the interior of the nerve cell. The central nervous depressant action of neostigmine, a quaternary ammonium compound with negligible lipoid solubility, might be due to its inability to pass through the nerve cell membrane. Thus the hypothesis suggested that intracellular A.Ch. was stimulant to nerve cells, whereas extracellular A.Ch. was depressant. In this connection it is interesting to note that DFP is lipoid soluble, and, as described above, it is a central convulsant.

The actions of DFP on the electroencephalogram (EEG) in man have been described by Grob *et al.* (1946). After a few days administration of 1–2 mg. of DFP daily there was usually some increase in electrical activity in the EEG (greater variations in potential, increased frequency of β waves, and the appearance of high voltage waves at frequencies of 3–6 per sec.). These signs were usually associated with central nervous symptoms such as excessive dreaming, nightmares, insomnia and restlessness. It was reported that the EEG changes were inhibited by intravenous injection of 1.2 mg. of atropine, and that this drug could also delay the onset of central nervous symptoms.

General Actions of HETP and TEPP

The condensed alkyl phosphates HETP and TEPP were introduced in Germany during the war as insecticides (Schrader, 1947) and it has been shown by Ludvik and Decker (1947) that they are

many times more potent than nicotine against aphides. They are powerful inhibitors of cholinesterase, HETP being as potent as physostigmine, neostigmine and DFP against the "true" enzyme ; TEPP is about four times as powerful (Burgen, 1948).

These compounds produce similar symptoms and signs to those described following DFP administration. Burgen *et al.* (1949) showed that these substances potentiated the effects of vagus stimulation on the heart, chorda tympani nerve stimulation on salivary secretion, and preganglionic sympathetic nerve stimulation on the nictitating membrane response. They also enhanced the actions of injected A.Ch. on these organs, hence their effects could well be due to their anti-cholinesterase properties. HETP and TEPP have a longer duration of action than physostigmine and neostigmine, but shorter than that of DFP.

Chennells *et al.* (1949) have studied the actions of HETP and TEPP on neuromuscular transmission and on the central nervous system. Both substances increased the muscular tension response to motor nerve stimulation and produced repetitive electrical potentials (cf. DFP). They also antagonized the action of curare. On the central nervous system they were both convulsant (TEPP is lipoid soluble, though less so than DFP) and they facilitated and prolonged such reflexes as the knee jerk, flexor and extensor reflex.

Grob and Harvey (1949) have shown that arterial injection of TEPP (0.25–2.5 mg.) produced in normal persons marked muscular fasciculations and weakness, which lasted about twice as long as after neostigmine. The electromyographic changes were similar to those seen with neostigmine and DFP.

The Actions of Anti-cholinesterase Drugs in Myasthenia Gravis

Myasthenia gravis is a disease characterized by a gradually developing weakness of the muscles of the body. The muscles supplied by cranial nerves, e.g. levator palpebri superioris, external ocular, facial, palatal, pharyngeal, laryngeal and neck muscles are usually the first to be affected, then limb muscles, abdominal muscles, intercostals and the diaphragm are involved in that order. In normal subjects these muscles show the same order of susceptibility to the action of curare.

The cause of myasthenia gravis is not yet clear, but on the

theory that A.Ch. is the chemical transmitter at the neuromuscular junction the following possibilities must be considered :—

(i) There might be an increase in cholinesterase activity in the affected muscles. Evidence in support of this view cannot be regarded as satisfactory.

(ii) There might be a diminished release of A.Ch. at motor nerve endings. Harvey and Lilienthal (1941) reported that injection of A.Ch. into the brachial artery of patients with myasthenia gravis produced a violent contraction of the flexor muscles of fingers, hand and wrist. This response was quite different from the paralytic effect of arterial A.Ch. in normal subjects (Harvey *et al.*, 1941). These authors therefore suggested that in myasthenia gravis there was diminished A.Ch. liberation at motor nerve endings, and that the muscle was thereby sensitized to the action of A.Ch. However, Wilson and Stoner (1947) and Acheson *et al.* (1948) observed muscular contractions after arterial injection of A.Ch. as frequently in normal subjects as in myasthenic patients ; the evidence to support the hypothesis of reduced liberation of A.Ch. at motor nerve endings is thus very doubtful.

(iii) The close resemblance between the paralysis of myasthenia gravis and that produced by curare was noted by Walker (1934), who later (1938) provided some indirect evidence that active contractions of muscles in myasthenic patients caused the liberation into the blood stream of a curare-like substance ; Wilson and Stoner (1944) claimed that the blood of myasthenics (after muscular activity) contained a substance capable of depressing neuromuscular conduction in the frog's sciatic nerve-gastrocnemius preparation. This latter work has been criticized on experimental grounds, but the possibility that some curare-like agent is responsible for myasthenic paralysis would appear to be the most fruitful line for future investigations. This view, which is compatible with the well known hyper-sensitivity of myasthenic patients to the paralysing action of curare, is supported by some recent observations of Buchthal and Engback (1948). These authors found that myasthenic patients showed a raised threshold for the motor response produced by arterial injection of A.Ch.

Whatever the nature of the abnormality in myasthenia gravis, there is no question that anti-cholinesterase drugs can restore muscular power in a most remarkable way. This was first shown by Walker (1934) with physostigmine, and subsequently with

neostigmine which, because of its fewer side actions, became the drug of choice in this condition. More recently it has been shown that other anti-cholinesterase drugs such as DFP, HETP, and TEPP can also restore muscular power in this disease.

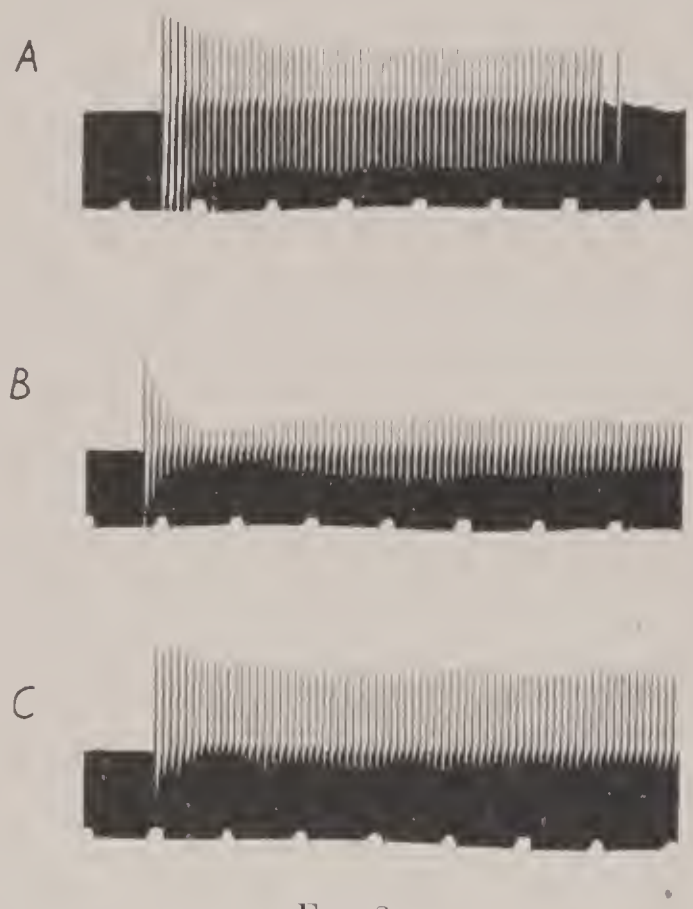


FIG. 2

ELECTROMYOGRAM IN MYASTHENIA GRAVIS (see text)

A. Normal Control.
B. Myasthenia Gravis, before injection of neostigmine.
C. Myasthenia Gravis, 30 minutes after injection of neostigmine.

(After Harvey and Masland, 1941.)

Actions of Anti-cholinesterase Drugs on the Electromyogram in Myasthenia Gravis

Harvey and Masland (1941) have studied the electrical changes in the hypothenar muscles following stimulation of the ulnar

nerve in myasthenic subjects, surface electrodes being used both for stimulation and recording. The results were as follows: (1) the action potential produced by a single maximal nerve volley was of normal size and contour, (2) when pairs of stimuli were applied (interval of e.g. 20–100 msec. between stimuli), the second potential response was smaller in magnitude and shorter in duration than the first. This abnormal response suggests that the second stimulus could not excite all the muscle fibres. (3) The most characteristic abnormality of the myasthenic electromyogram was the response to repetitive nerve stimulation at high rates, e.g. 50/sec. In normal subjects the muscle potentials were of constant amplitude throughout the tetanus, but in myasthenics the tetanic muscle potentials diminished progressively in size, till at the tenth response the amplitude was about 40 per cent of that of the first in the series (Fig. 2).

Twenty to thirty minutes after subcutaneous injection of neostigmine in a myasthenic patient all electromyographic abnormalities were completely eliminated.

Harvey *et al.* (1947) recorded a similar electromyographic response after intra-arterial injection of DFP in myasthenia gravis, the maximum effect occurring four hours after injection.

Intra-arterial Injection of Anti-cholinesterase Drugs in Myasthenia Gravis

Neostigmine. The effects of intra-arterial injection of neostigmine in normal subjects have already been described. In myasthenics, injection of 0.5–3 mg. of neostigmine into the brachial artery was found by Harvey and Lilienthal (1941) to produce immediate restoration of muscular power in the injected region, and 2–5 minutes later increased power in the muscles of the rest of the body. The maximum effect was seen in 5–10 minutes and the total duration was 3–5 hours. There were usually no muscular fasciculations such as follow intra-arterial injection of neostigmine into normal muscle.

DFP. Harvey *et al.* (1941) showed that arterial injection of DFP (0.5–1.5 mg.) produced an increase in muscular power (maximal in 15 minutes), but no fasciculations, in the injected area. Unlike neostigmine, however, arterial injection of DFP had no effect on muscular power in other parts of the body; this is probably due to rapid inactivation of DFP by an enzyme present

in the blood (Mazur, 1946). The prolonged inactivation of cholinesterase by DFP probably accounts for its amazing duration of action on muscular power, up to many weeks after a single arterial injection (compared with about six hours for neostigmine).

Harvey and his colleagues recorded some interesting responses when both neostigmine and DFP were given by arterial injection to the same myasthenic patient. If DFP was given first, the effect of a subsequent injection of neostigmine was either to increase muscular power still further (when the DFP improvement was only partial) or to decrease muscular power as in a normal subject. Thus DFP followed by neostigmine appeared to have additive effects. If, however, neostigmine was given first, a subsequent injection of DFP, at the height of the neostigmine response, had no effect whatever, either on intensity of response, or on duration of action. In fact the response was identical with that produced by neostigmine alone, a finding which suggests that this drug in some way prevents the combination of DFP with cholinesterase. Koelle (1946) has shown that neostigmine reduces the cholinesterase inhibiting action of DFP *in vitro*, and Koster (1946) has found that physostigmine has a protective action against DFP poisoning in cats. The mechanisms of these actions have been discussed by Burgen (1949).

TEPP. Grob and Harvey (1949) found that arterial injection of TEPP in myasthenic patients produced an increase in muscular power (without fasciculations) which was maximal within ten minutes of the injection. As with neostigmine, the increase in power was not confined to the injected region. TEPP also restored to normal the electromyographic response to ulnar nerve stimulation at 10–50 cycles/sec. As with DFP, neostigmine blocked these actions of TEPP.

Therapeutic Effects of DFP in Myasthenia Gravis

Comroe *et al.* (1946) gave DFP (made up in peanut oil) to seven patients with myasthenia gravis. Intramuscular injection of 2–3 mg. of DFP produced effects for 24 hours. Three of the patients showed considerable subjective and objective improvement, two showed little or no benefit, and in two the effects were obscured by spontaneous variations in the course of the disease. In one patient after treatment with DFP for one month, when the plasma cholinesterase level was zero and the red cell cholinesterase value

was 14 per cent of normal, there was extreme muscular weakness which was rapidly and markedly relieved by injection of 1 mg. of neostigmine methyl sulphate. These authors were of the opinion that neostigmine has some direct stimulant action on striped muscle in addition to any effect due to its anti-cholinesterase properties (cf. Riker and Wescoe, 1946).

They also suggested that DFP and neostigmine might be distributed differently in the body and that this might be related to the lipoid solubility of DFP, and the water solubility of neostigmine (cf. Schweitzer *et al.*, 1939).

Harvey and his colleagues (1946) administered DFP in daily doses of 0.5–2 mg. to ten patients with myasthenia gravis. In nine of the ten patients DFP was regarded as inferior to neostigmine, though it did allow some reduction of neostigmine dosage; its one advantage was that owing to its prolonged action, it produced greater strength in the mornings than occurred with neostigmine alone. In no case did the maximal beneficial effects of DFP+neostigmine exceed those of neostigmine alone. DFP produced marked side effects on the central nervous system and on the gut.

It is clear from these results that DFP is not a satisfactory drug in the treatment of myasthenia gravis.

HETP in Myasthenia Gravis

Westerberg and Luros (1948) gave HETP to reinforce the effects of neostigmine in four patients with myasthenia gravis. They gave intramuscular injections of up to 0.86 mg. kg. body weight at 3–4 day intervals, and in this way the prolonged action of the drug helped to maintain muscular power at a much more satisfactory level than with neostigmine alone. The toxic actions of HETP were generally similar to those of DFP and included the typical muscarinic, nicotinic and central nervous effects.

TEPP in Myasthenia Gravis

Burgen *et al.* (1948) have described the effects of TEPP in three patients with myasthenia gravis. TEPP is more suitable than HETP for therapeutic use as it is a pure substance. It was found to be about $\frac{1}{3}$ to $\frac{1}{2}$ as potent as neostigmine but its action was much more prolonged (up to about 48 hours). Repeated doses produced cumulative effects which allowed a more constant effect on

muscular power, but at the same time predisposed to visceral and central nervous toxic effects resembling those of neostigmine. One of the patients had insomnia and nightmares (cf. DFP) but these were readily controlled by barbiturates (though not by atropine). TEPP was effective by mouth, 10 mg. being equivalent to about 100–150 mg. of neostigmine by this route. After initial administration of TEPP by injection, an oral maintenance dose of 8–18 mg. daily was established. Further unpublished observations have shown that TEPP is definitely less effective than neostigmine in some cases, particularly where muscles supplied by cranial nerves are predominantly or solely involved. TEPP may also sometimes depress respiration by mechanisms which are not yet clear.

Grob and Harvey (1949) have also reported on the therapeutic use of TEPP in myasthenia gravis. They found that TEPP could produce as much increase in muscular strength as neostigmine. After an initial build-up by oral administration or intramuscular injection, they gave a daily maintenance dose of about 16 mg. by mouth or 4.8 mg. by injection (in both cases in divided doses). The margin of safety with TEPP was very small; over-dosage produced muscular weakness as well as the side effects previously mentioned. Grob and Harvey found TEPP preferable to neostigmine in 9 of 12 patients.

To summarize, the actions of TEPP in myasthenia gravis are sometimes as good as those of neostigmine, and when it is effective its prolonged action maintains muscular power much more evenly than with neostigmine. However, its actions are not so certain as those of neostigmine, and it has a very small margin of safety.

DFP on Intestinal Motility. Action in Abdominal Distention

Grob *et al.* (1947) recorded intestinal activity in normal subjects by means of a balloon attached to a Miller-Abbott tube. 1–3 mg. of DFP intramuscularly produced a marked increase in motility of small and large gut, commencing in 1 hour and lasting 2–5 hours. Atropine antagonized this effect of DFP (it also prevents this action of neostigmine, HETP and TEPP). 24–40 hours after a single injection of DFP there was marked sensitization to the gut-stimulating actions of neostigmine, vasopressin, morphine and smoking. This sensitization lasted 1–3 weeks.

DFP was found to be very effective in 64 cases of abdominal

distension due to various causes. In the milder cases a single injection of 2 mg. produced flatus in 1–2 hours and a bowel action in 2–8 hours. More severe cases responded to DFP followed by neostigmine or vasopressin, or to repeated injections of 1 mg. of DFP at 12 hourly intervals.

Quilliam and Quilliam (1949) have also recorded the beneficial use of DFP in postoperative paralytic ileus, and Arneil and Douglas (1949) have described its effectiveness in removing flatus.

Grob and Harvey (1949) found TEPP much less active than DFP on the bowel.

DFP on the Eye. Use in Glaucoma

Leopold and Comroe (1946) have studied the actions of DFP on the normal eye in man, and have made use of its prolonged effects in the treatment of patients with glaucoma.

Normal Eye. The instillation of a drop of 0.1 per cent DFP in water into the conjunctival sac of the normal eye produced *constriction of the pupil* in 5–10 minutes, with a peak action in 15–20 minutes, and a total duration of action of 6–27 days (0.1 per cent DFP was more potent than 1 per cent physostigmine salicylate or 5 per cent neostigmine bromide). *Intraocular tension* began to fall 5 minutes after application of DFP, the maximal reduction was seen at 24 hours, and tension was normal again in eight days.

Glaucoma. One daily application of 0.05–0.1 per cent DFP was as effective as, or better than, four daily applications of physostigmine or pilocarpine. 0.1 per cent DFP produced no signs of conjunctival irritation, but headache, eyeache and visual blurring, attributable to ciliary spasm, frequently occurred. No systemic effects were recorded. Marr (1949) found that in glaucoma TEPP acted like DFP, but was more irritant to the eye.

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CHAPTER 2

CURARE, MEPHENESIN (MYANESIN), AND TETRAETHYLAMMONIUM SALTS

In this chapter the neuromuscular blocking actions of *curare* and other drugs, and the ganglionic blocking actions of *tetraethylammonium* salts will be discussed. For convenience the actions of *mephenesin* (*myanesin*) will also be described here.

Natural Sources of Curare and Related Drugs

McIntyre (1947) has given an excellent review of the history of the preparation of curare as an arrow poison in South America. The obscurity of the botanical origins of the various curares still makes difficult the interpretation of pharmacological investigations of the crude curare preparations. Boehm's classification of the curares according to the type of container (calabash-, pot- and tube-curare) was certainly helpful, but what is wanted now is knowledge of the exact botanical sources of the numerous curare alkaloids. d-tubocurarine Cl, which was first isolated from tube-curare by King (1935), was subsequently found in crude curare prepared from the Peruvian plant *Chondrodendron tomentosum* (Wintersteiner and Dutcher, 1943). Even in this instance, however, the issue is not yet quite clear, as King, at a meeting of the Chemical Society in April 1948, reported that the stems from one sample of Ch. tomentosum contained d-tubocurarine Cl, whereas those from another sample contained *l*-tubocurarine Cl.

Chemical Structure in Relation to Curare-like Action

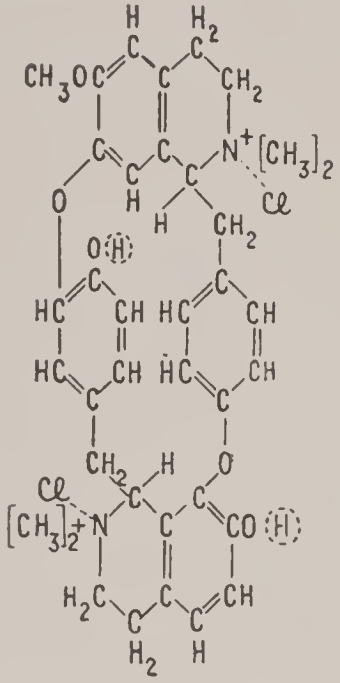
This subject has been extensively reviewed by Craig (1948) and some of the points in his review will be briefly discussed.

(1) Peripheral curare-like paralysis is commonly associated with onium compounds, of which quaternary ammonium salts are the most effective. Ing (1936) has discussed this group of substances.

(2) A few nitrogen compounds which are not quaternary have

a weak curare-like action. In general the conversion of such a compound to a quaternary compound greatly increases the curare-like action. For example, Harvey (1940) showed that quinine methochloride (quaternary) had a stronger curariform action than quinine (tertiary).

(3) In the quaternary ammonium compounds of the aliphatic series, the length of side chain has an extraordinary effect on



d-tubocurarine Chloride

The O-dimethyl ether is formed by substitution of CH_3 groups in the two ringed H atoms

Erythroidine has the empirical formulæ $C_{16}H_{19}NO_3$. It contains a tertiary N common to two rings, a CH_3 group, two olefinic double bonds and a lactone ring. It contains an indole nucleus

curariform activity. The tetramethyl- and tetrabutyl-compounds are about equally potent, tetrapropyl- is weaker, and tetraethyl- has practically no curare-like action at all. These differences may be related to such factors as ionic volume (Ing, 1936) or electron distribution (Holmes *et al.*, 1947).

(4) In the quininium series increasing the length of side chain from CH_3—to C_5H_{11}—produces a progressive increase in curariform activity.

(5) The erythrina alkaloids which contain tertiary N have a powerful curare-like action. Conversion to the quaternary compound abolishes curare-like activity. This is exceptional. Dihydroerythroidine is more powerful than erythroidine.

(6) *The Curare Alkaloids.* The curare alkaloids are *bis*-benzyltetrahydroisoquinoline compounds. Methylation of the two free OH groups in d-tubocurarine Cl enhances curare-like activity about 10 times. Ethylation decreases, and butylation abolishes the action. *l-curarine dimethiodide* is nearly as potent as d-tubocurarine Cl ; methylation of its two free OH groups increases its potency about $3\frac{1}{2}$ times.

d-chondrocurarine Cl is about three times as potent as d-tubocurarine Cl.

d-N-methylisochondrodendrine is 1/20 as potent as d-tubocurarine Cl.

(The above potency comparisons are based on the rabbit head drop test.)

A compound closely related chemically to d-tubocurarine, N-methyloxyacanthine, has been studied by Marsh *et al.* (1949), who found that it was about half as potent as d-tubocurarine, and that it also had a weak atropine-like action.

Methods for Testing Curare-like Actions of Drugs

Since the introduction of curare as an adjunct to general anæsthesia by Griffith and Johnson (1942) the search for new compounds with a greater safety-margin than d-tubocurarine has created a need for accurate methods of assaying curare-like action, and the following are the three most important procedures which have been employed.

(1) *Rabbit Head Drop.* This method was introduced by Holaday (Bennett, 1940). It depends on the weakness of the neck muscles produced after intravenous injection of the drug in unanæsthetized rabbits ; the head drops forward and cannot be raised on stimulation of the animal. d-tubocurarine Cl produces "Head Drop" in 50 per cent of rabbits in a dose of about 0.15 mg./kg. ($H.D._{50}$).

(2) *The Rat Phrenic Nerve-diaphragm Preparation.* The isolated rat phrenic nerve-diaphragm preparation has been extensively used in this country (Bülbring, 1946 ; Chou, 1947). The drug to be tested is introduced into the bath and its effect on the

contraction of the diaphragm in response to phrenic nerve stimulation at 5/min. is observed. Repeated measurements of curare-like activity can be made on one preparation. Phrenic nerve-diaphragm preparations from kittens, very young rabbits and other animals may also be used (Trevan and Mogey, 1948).

(3) *Mouse Method.* In this method (Skinner and Young, 1947) the curare preparation is injected intravenously in the tail vein of mice, and the onset of paralysis is indicated when the animals can no longer hold on to a slowly rotating cylinder covered by a wire mesh. Normal mice maintain their grip on the cylinder indefinitely.

Recent Developments Concerning the Mode of Action of Curare

The classical studies of Claude Bernard in the middle of the last century localized the paralytic action of curare to some point between the motor nerve and skeletal muscle. Langley (1909) suggested that curare combines with "receptive substances" in the region of the myoneural junction, and thereby prevents their being acted upon by nerve stimuli. These older facts and theories can be readily correlated with newer developments in the fields of chemical transmission and electrophysiology.

Dale *et al.* (1936), in experiments on the perfused cat's tongue, showed that after the production of full paralysis by curarine, hypoglossal nerve stimulation caused the liberation into the perfusing fluid of the same amount of acetylcholine as that recovered from normal muscle. Thus a paralysing dose of curarine did not inhibit the release of acetylcholine by nerve impulses at motor nerve endings. Brown and Feldberg (1936) also showed that curarine produced an analagous type of blocking action on transmission through the superior cervical sympathetic ganglion; when the ganglion was paralysed by curarine, preganglionic nerve stimulation could still liberate normal quantities of acetylcholine. The mode of action of curarine in blocking neuromuscular and ganglionic transmission thus resembles that of atropine at postganglionic parasympathetic nerve endings.

Brown *et al.* (1936) found that curarine more readily inhibited the contraction of the cat's gastrocnemius muscle evoked by close arterial injection of acetylcholine, than the contraction produced by motor nerve stimulation. Buchthal and Lindhard (1942), in

their studies of single motor end plate responses in the lizard, showed that curarine first blocks both the action of acetylcholine and the effect of nerve stimulation, while K ions remain effective in producing muscular contraction. Later the effect of K is also abolished.

The actions of physostigmine and curarine on the electrical accompaniments of neuromuscular conduction have been very fully investigated by Eccles *et al.*, who have published many papers on the effects of these drugs on the end-plate potential (e.p.p.) (e.g. Eccles *et al.*, 1941 ; 1942 ; Eccles and Kuffler, 1941 ; Eccles and MacFarlane, 1949 ; Kuffler, 1942 ; see also chapter 1). Physostigmine increased the magnitude and duration of the e.p.p. produced by a motor nerve volley, but had no effect on the subsequent spike potential associated with muscular contraction (cat soleus or frog sartorius). Normally the e.p.p. is dissipated by the spike potential and lasts only a few millisec. The action of physostigmine was to prolong the e.p.p. to 50–100 millisec., and to increase its magnitude so that repetitive muscle spikes occurred. Physostigmine also produced a secondary "slow-wave" of end-plate negativity which might persist for several seconds. These end-plate potential changes underlie the repetitive electrical discharge and the increased mechanical tension which are developed after physostigmine or other anti-cholinesterase drugs. Curarine antagonized all these effects of physostigmine.

Curarine, acting alone, produced the following effects on the potentials at end-plate and muscle fibre.

(1) Diminution in size of the e.p.p.
(2) No change in latent period, but shortening of duration of e.p.p.
(3) Delay in the onset of spike potentials.
(4) Eventual abolition of spike potentials.

The neuromuscular blocking action of curarine is finally associated with a reduction in size of e.p.p. to below the critical level required for the firing off of the spike potential. Curarine would appear to oppose the depolarizing action of acetylcholine at the end-plate, an antagonism possibly related to the fact that both substances are quaternary ammonium compounds which would tend to become attached to the same end-plate receptors ; this would be an example of "receptor competition."

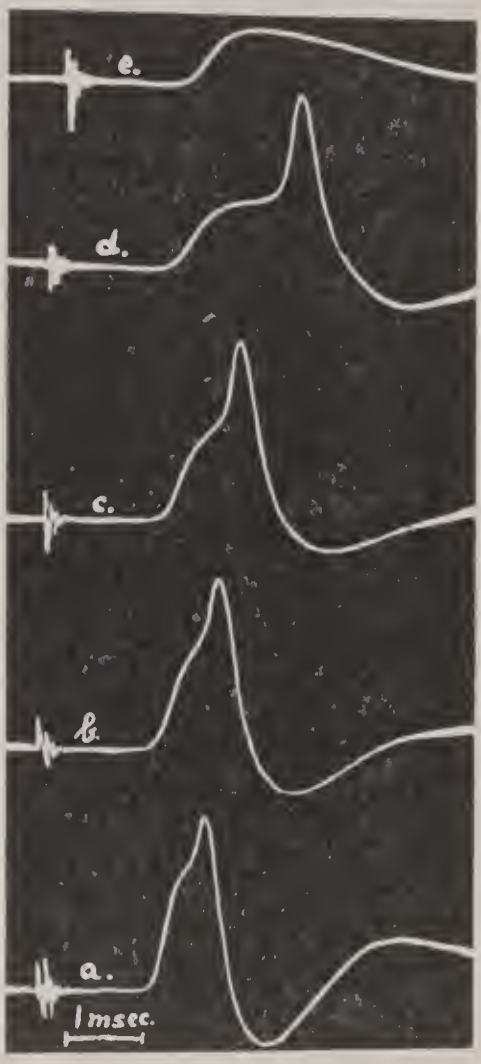


FIG. 3

CURARINE ON END-PLATE POTENTIAL (EPP.)

Records taken at end-plate region in frog muscle, *a*, before application of curarine; *b*, *c*, and *d*, show diminution of the initial EPP as curarine action increases. Note progressive lengthening of spike latent period; *e*, pure EPP with no spike potential, i.e. complete neuromuscular block.

(After Kuffler, 1942.)

Curare Paralysis in Relation to Rate of Motor Nerve Stimulation. The action of curarine on neuromuscular conduction is related to the frequency of nerve stimulation. Briscoe (1936), using the cat's femoral nerve-quadriceps preparation, showed that although after curarine the magnitude of contraction increased in direct

proportion to the frequency of stimulation (up to 240/sec.), at the higher frequencies the contraction became merely a brief twitch. Thus the action of curarine was to prevent the sustained contraction normally produced by tetanic stimulation. Brown (1938) showed that curarine caused a rapid decline in height of muscle spike potentials after motor nerve stimulation at 50/sec. in the cat, and Harvey and Masland (1941) observed a similar effect in man after "curarization" by quinine methochloride. It should be

FIG. 4

TUBOCURARINE-NEOSTIGMINE ANTAGONISM

Record shows contraction of the isolated rat diaphragm in response to stimulation of the phrenic nerve at 10/min. (method as described by Bülbring, 1946).

At 1st ↑ 100 μg. of d-tubocurarine Cl were added to the bath. Note the rapid decrease in height of contraction.

At 2nd. ↑ 150 μg. of neostigmine methyl sulphate were added to the bath (without previous washing). Note the rapid restoration of the force of muscular contraction.

(Tracing obtained by Dr. A. S. V. Burgen and Dr. L. J. Zatman.)

emphasized, however, that this failure to sustain contraction is quite different from the normal effect of fatigue.

Antagonists to Curare

The theory of chemical transmission by acetylcholine at motor nerve endings, and the demonstration of the mode of action of curare, naturally suggest that the latter drug could be antagonized by acetylcholine itself, or by substances which increase the local concentration of acetylcholine at the motor end-plate region. The anti-cholinesterase drug physostigmine was first shown by Pal (1900) to have an anti-curare action, and the similar effect of neostigmine has been frequently recorded (e.g. Bülbring and Chou, 1947) (see Fig. 4). The anti-curare actions of a number of drugs have been compared by Chase *et al.* (1947) using the rabbit head drop test. Neostigmine, in a dose of 0.05 mg./kg. intravenously, increased the head drop dose of tubocurarine two and a half times; 0.075 mg./kg. of neostigmine was no more effective than 0.05 mg./kg., and 0.122 mg./kg. of neostigmine itself produced head drop. Among the anti-cholinesterase drugs comparison of anti-curare activity on a molar basis gave these figures for potency.

$$\text{Neostigmine} = 1,\ \text{Physostigmine} = \tfrac{1}{2},\ \text{DFP} = \tfrac{1}{8}.$$

These substances presumably act by increasing the concentration of acetylcholine so that it can overcome the threshold raising action of curarine at the motor end-plate. It must, however, be remembered that too high concentrations of acetylcholine are themselves depressant, so that if the curarine effect were very great it might be impossible to overcome it with acetylcholine at all. Thus in cases of curare overdosage, anti-cholinesterase drugs are of doubtful value. Chase *et al.* (1949) showed that in rabbits large doses of DFP (e.g. 0.6 mg./kg.) reduced the dose of tubocurarine required to produce head drop, and indeed might themselves produce chronic intermittent head drop. They noted a further interesting point, that after such doses of DFP, neostigmine could sometimes precipitate a prolonged head drop, a finding of importance in relation to the clinical observations of Burgen *et al.* (to be published) that neostigmine was sometimes quite ineffective in myasthenic patients who had previously been treated with TEPP.

The anti-curare action of potassium salts was demonstrated by Wilson and Wright (1936), who showed that intra-arterial injection of KCl produced an immediate and very marked increase in muscular power in cats in which curarine had reduced the response to about 20 per cent of normal. The recovery of muscular power following intra-arterial neostigmine was more gradual than that produced by KCl.

It has been claimed that guanidine antagonizes the actions of curare, but Bülbring and Chou (1947) found this effect to be very feeble on the rat-phrenic nerve-diaphragm preparation.

It is very fortunate, from the clinical point of view, that the muscle paralysing dose of curare has no other serious toxic effects. Many times the dose necessary to produce respiratory paralysis can be tolerated provided, as Sir Benjamin Brodie showed in 1812, that artificial respiration is maintained until muscular power returns. To overcome the difficulties arising from possible bronchiolar spasm the artificial respiration should be of the positive pressure type such as anæsthetists can so readily use by pressure on the anæsthetic bag.

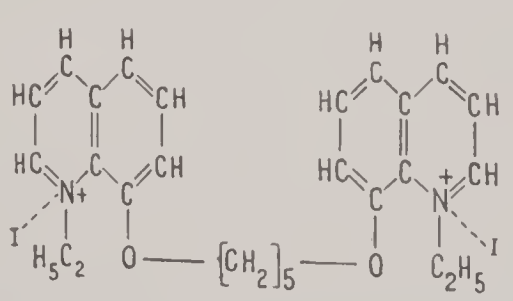
Actions of Synthetic Curariform Agents

The actions of a number of synthetic curariform agents have been reviewed by Bovet and Bovet-Nitti (1948) and by Cheymol (1949).

Bovet *et al.* (1947 ; 1949), in studying simplifications of the tubocurarine molecule, found that *bis* (8-quinolyloxy) $\alpha : \omega$ pentane diethiodide (3381 RP) and *bis* (2-dimethylaminophenoxy) $\alpha : \omega$ pentane dimethiodide (3565 RP) were both nearly as potent as tubocurarine, and that their effects were antagonized by physostigmine. Compound 3381 R.P. also had weak anti-cholinesterase actions.

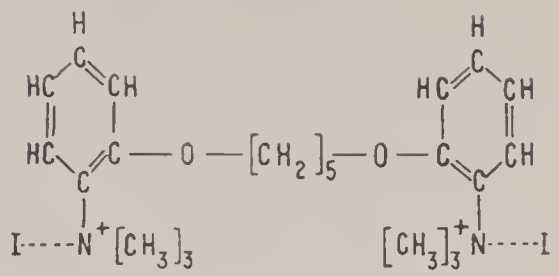
"*Bovet Compounds*"

3381 R.P.



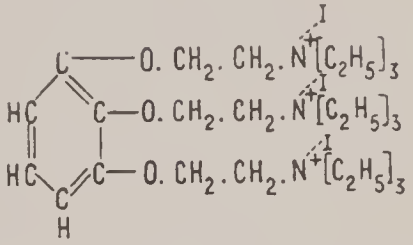
Bis (8-quinolyloxy) $\alpha : \omega$ pentane diethiodide

3565 R.P.



Bis (2-dimethylaminophenoxy) $\alpha:\omega$ pentane dimethiodide

Compound R.P. 3697
("Flaxedil")



1:2:3-*Tris* (β-diethylaminoethoxy)-benzene triethiodide

Wien (1948) has shown that another of Bovet's compounds, 1:2:3-*tris* (β-diethylaminoethoxy)-benzene triethiodide (3697 RP "Flaxedil") is also a powerful curariform agent with a very weak ganglionic blocking action; its effects are reversed by physostigmine and neostigmine.

Bovet *et al.* (1948) have reported on the curare-like actions of the di-choline ester of terephthalic acid and related compounds. They are weaker than tubocurarine, but their most important feature is their brief duration of action, which is probably due to the ease with which the ester bond is split *in vivo*.

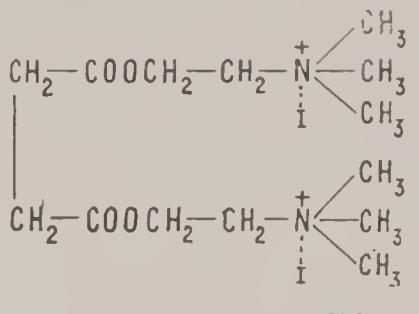
Bovet and Bovet-Nitti (1949) have also shown that succinyl choline and the closely related *bis*-(β-dimethylaminoethyl) succinate diethiodide (362 I.S.) are powerful curare-like agents; the latter is about as potent as tubocurarine, though shorter acting, and since it does not lower the blood pressure like succinyl choline it has been considered suitable for clinical trial.

Polymethylene Bistrimethylammonium Salts

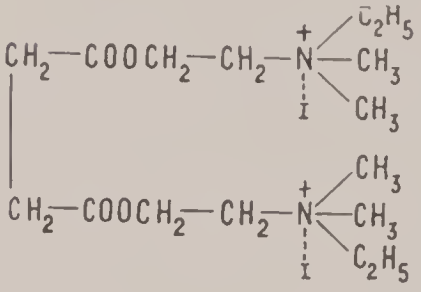
General structure $(CH_3)_3N^+(CH_2)_n{}^+N(CH_3)_3.2\ I^-$

In Pentamethonium iodide (C_5), n = 5

In Decamethonium iodide (C_{10}), n = 10



Succinyl Choline Iodide



362 I.S.

It will be noted that the spacing between the nitrogen groupings in both these compounds is comparable to that in decamethonium iodide

Bovet and Bovet-Nitti (1948) discuss earlier work with compounds of this group containing up to 5 methylene groups, but these substances had very weak curare-like actions. Detailed studies on the actions of the polymethylene *α, ω-bis*trimethylammonium salts $(CH_3)_3\ N^+(CH_2)_n\ N^+(CH_3)_3 2\ I^-$ have recently been reported by Barlow and Ing (1948) and by Paton and Zaimis (1948 ; 1949). The results of experimental work on these compounds may be summarized thus :—

(1) Decamethonium iodide (C_{10}) paralyses transmission at the neuromuscular junction. Pentamethonium iodide (C_5) paralyses transmission at the ganglionic synapse. Adjacent members of the series have similar properties in less degree.

(2) Decamethonium iodide produces neuromuscular block in very small doses, e.g. in the chloralosed cat, 30–40 μg./kg. i.v., or 1 μg./kg. by close arterial injection, are effective.

(3) A striking feature of the action of decamethonium iodide in cats is the relatively wide margin between the dose required to

paralyse leg muscles and that required to paralyse respiratory muscles. With tubocurarine the ratio $\frac{\text{dose required to paralyse respiration}}{\text{dose required to paralyse limb muscles}} = 1.25$; with decamethonium iodide this ratio=2.2.

(4) Decamethonium iodide differs from d-tubocurarine in certain respects :—

(*a*) It has an initial stimulating action on skeletal muscle when given by close arterial injection. It also has an anti-cholinesterase action, especially against the "true" enzyme.
(*b*) After partial paralysis by decamethonium iodide, motor nerve tetanus produces a well-sustained contraction.
(*c*) The action of decamethonium iodide is not antagonized by physostigmine or neostigmine. It is however reversed by pentamethonium iodide, when the latter is administered in 10–100 times the dose of decamethonium iodide.
(*d*) Decamethonium iodide has less ganglionic blocking action than tubocurarine.
(*e*) Decamethonium iodide has weaker histamine and heparin-liberating actions than tubocurarine.

(5) Studies in various animal species show that the action of decamethonium iodide is greatest in the cat and in man. The actions in man will be discussed later.

(6) Decamethonium iodide is easily synthesized, and requires no biological standardization.

(7) Pentamethonium iodide produces ganglionic block without diminishing the release of acetylcholine from preganglionic nerve terminals. It has no anti-cholinesterase activity, no muscarine-like actions, and no stimulating actions like nicotine.

Paralysing Action of Tubocurarine in Normal Man

The sequence of paralysis of the muscles by curare has already been mentioned in comparing the effects of this drug with the order of involvement of muscles in myasthenia gravis (Chap. 1). The approximate sequence is—muscles supplied by cranial nerves, limb muscles, abdominal muscles, intercostals, and finally the diaphragm. This may be illustrated by experiments recorded by Prescott *et al.* (1946) in which Dr. Prescott was himself the subject.

d-tuboeurarine Cl was injeeted intravenously and the effeets were as follows : —

1–2 mg. produeed only diplopia, from an effeet on the extrinsie ocular museles.

10 mg. produeed diplopia, ptosis, strabismus, and a feeling of weakness, and partial loss of power in other museles.

20 mg. The above effeets oeeurred in less than one minute, and there was paralysis of faee and neek museles in two minutes. There was then rapid spread of paralysis to the larynx, limbs, abdomen and intereostals ; the tidal air was reduced from 750 to 500 ml. but the respiratory rate was unehanged. Reeovery of power oeeurred in the reverse order and began in about 15 minutes, the eye signs taking longest to disappear (3–4 hours).

30 mg. Within three minutes there was complete paralysis, ineluding the intereostals. At five minutes the tidal air had fallen from 750 to 115 ml. and artificial respiration was neeessary owing to the inadequaey of the rapid shallow diaphragmatie eontraetions. (The eentral nervous effeets are eonsidered elsewhere.) Museular power returned in 30–40 minutes, but diplopia and ptosis were still present at four hours. After six hours there was nothing abnormal exeept a feeling of tightness in the ehest whieh lasted several days.

Variations in Sensitivity to Tubocurarine

Apart from the well-known hypersensitivity of myasthenie patients to tuboeurarine (Bennett and Cash, 1943), some apparently normal persons may reaet exeessively to this drug. For example, Gray and Halton (1948) observed eomplete museular paralysis with respiratory arrest after injeetion of 2 mg. of d-tuboeurarine Cl into an unanæsthetized patient. These authors, and others (Salan and Carmiehael, 1948) have reeorded deaths from the usual doses of tubocurarine. It has therefore been suggested that the response to a test dose of 5mg. of d-tuboeurarine Cl be observed before the full amount is administered.

Actions of Erythroidine and Quinine Methochloride in Man

Burman (1940) and Harvey and Masland (1941) have shown that intravenous injection of erythroidine or quinine methochloride produces eurare-like paralysis, associated with drowsiness

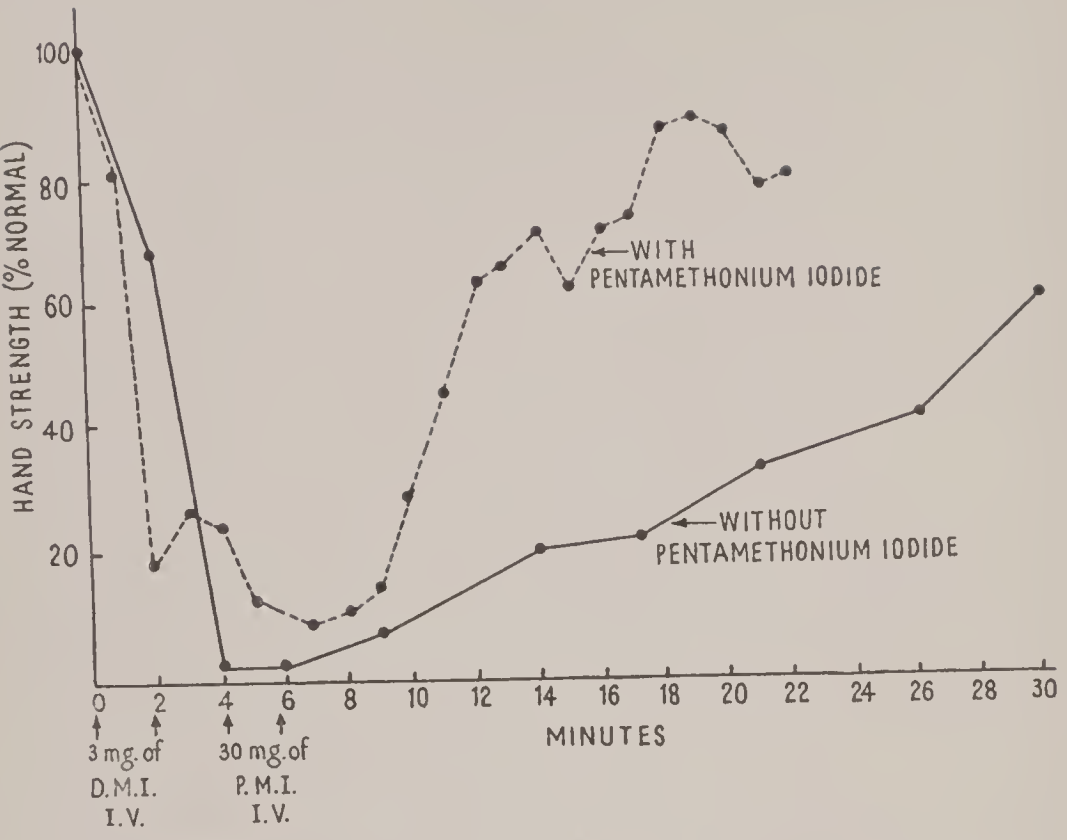


FIG. 5

ACTION OF DECAMETHONIUM IODIDE IN A NORMAL SUBJECT

The reduction in hand strength after intravenous injection of 3 mg. of decamethonium iodide is well seen. The more speedy recovery after injection of 30 mg. of pentamethonium iodide is also shown.

(After Organe, Paton and Zaimis, 1949.)

and other signs of central nervous depression. The blood pressure may be reduced, sometimes to a dangerous degree. These drugs are too toxic for therapeutic use.

Actions of Synthetic Curarizing Agents in Normal Man

Decamethonium Iodide

Organe *et al.* (1949) studied the effects of decamethonium iodide in three normal persons. They noted that 3 mg. intravenously produced a degree of paralysis roughly comparable with that produced in a conscious subject by 15–20 mg. of d-tubocurarine Cl. The reduction in hand strength, and the antagonism exerted by

pentamethonium- towards decamethonium iodide, are illustrated in Fig. 5. When abdominal muscular weakness was well marked vital capacity was reduced by about 35 per cent. No significant side effects were recorded with 3 mg. of decamethonium iodide. Grob *et al.* (1949) noted that intravenous injection of 2.5 mg. of decamethonium iodide produced almost complete paralysis of the muscles of the extremities with relatively slight weakness of the muscles concerned with swallowing, speech and respiration.

Organe *et al.* also observed the effects of intravenous injection of 20–40 mg. of pentamethonium iodide in the same subjects. There were signs of cutaneous vasodilatation, and orthostatic hypotension for up to 30 min. after injection.

R.P. 3697 ("Flaxedil")

Mushin *et al.* (1949) have investigated the actions of "Flaxedil" in conscious persons. Intravenous injection of 40–70 mg. produced complete paralysis of forearm and abdominal muscles without demonstrable decrease in pulmonary ventilation. Recovery from paralysis occurred in about 25 min., and could be greatly hastened by intravenous injection of neostigmine. The drug had no significant effect on blood pressure.

Curare and the Central Nervous System

The actions of curare on the central nervous system have been shown by many workers, some of whom have observed stimulant actions, and others have recorded depressant effects. Stimulation has most frequently been produced by direct application of curare or d-tubocurarine Cl to nervous tissue, and depression by intravenous injection of large doses. It is not proposed to discuss the conflicting experimental work on animals, which has been reviewed by McIntyre (1947), but an account will be given of some important observations in man. Dr. Prescott (Prescott *et al.*, 1946) described the sensation of respiratory muscle weakness after 30 mg. of d-tubocurarine as most terrifying, more particularly because he was unable to inform his colleagues about the obstruction in his larynx due to the collection of mucus. He began to lose consciousness before artificial respiration was begun, but this was probably due to asphyxia. At no time was there any analgesic or hypnotic action ; indeed no central effects ascribable to the drug *per se* were noted.

A similar lack of cerebral effects after intravenous injection of $2\frac{1}{2}$ times the respiratory paralysis dose of d-tubocurarine was recorded also by Smith *et al.* (1947) in an experiment on Dr. S. M. Smith. They observed no changes in the electroencephalogram, no mental disturbances and no alterations in sensation. In particular there was no analgesia. In this case artificial respiration with O_2 administration was quite adequate throughout.

A specific study of the possible analgesic action of d-tubocurarine was undertaken by Kellgren *et al.* (1946). Intravenous injection of 7.5 mg. of d-tubocurarine Cl produced weakness of facial, neck and limb muscles but there was no change in pain or touch sensations tested by von Frey's hairs and needles. There were also no cerebral changes as judged by mental arithmetic tests, etc.

There is thus no evidence for any significant central nervous action even when full paralysing doses of d-tubocurarine are given. Gray (1948) has described experiments on normal human volunteers which showed that d-tubocurarine Cl in no way potentiates the central depressant action of thiopentone. It may therefore be concluded that d-tubocurarine Cl has no effects of any practical importance on the central nervous system when administered intravenously as an adjunct to general anæsthesia.

Liberation of Histamine by Curare

West (1935) described the occurrence of bronchospasm in dogs and in man after administration of certain preparations of curare. The discovery by Alam *et al.* (1939) that curare or d-tubocurarine Cl causes the release of histamine from skeletal muscle suggests a possible mechanism for the production of bronchospasm. It is true that there is no evidence for release of histamine from the lungs, but that released from skeletal muscle could reach the lungs via the blood stream. Schild and Gregory (1947) showed that when d-tubocurarine and other curarizing agents were added to fluid perfusing dog's striped muscle, there was no close relation between curare-like potency and the power to release histamine. Silva and Schild (1949) have made quantitative studies of this action of tubocurarine on rat muscle, and have speculatively suggested that this compound might compete with histamine to combine with the receptors in muscle to which the latter is bound before its release.

Comroe and Dripps (1946) demonstrated that intracutaneous injection of Intocostrin or d-tubocurarine Cl in man produced large wheals and flares similar to those produced by histamine. Intra-arterial injection of these substances had the same effect. Grob *et al.* (1947) analysed these actions more fully, and showed that the skin responses were inhibited by the anti-histamine drug, pyribenzamine. The liberation of histamine from the skin into the circulation was demonstrated by a considerable increase in HCl secretion by the stomach after intra-arterial injection of d-tubocurarine Cl. Grob *et al.* also showed that intramuscular injection of tubocurarine produced a moderate stimulation of gastric secretion, whereas intravenous injection had no significant effect. It should be emphasized that histamine liberation occurs only when high concentrations of tubocurarine come into contact with tissues such as skin or muscle. One might expect that bronchospasm would be observed more frequently after intramuscular than after intravenous injection, except perhaps when the latter be given very rapidly.

Curare and the Circulation

Apart from the secondary effects due to asphyxia, curare produces very little effect on the circulation. It is generally agreed that there is no toxic action on the heart, and the electrocardiogram is unaffected by the use of tubocurarine in anæsthesia (Gray, 1948). Reports on the effect of tubocurarine on blood pressure are somewhat conflicting, but although a fall may follow rapid intravenous injection of large doses in animals, the usual dose in man has generally no significant effect.

Curare and Respiration

Curare influences breathing by its peripheral paralysing action on the respiratory muscles, and it may sometimes cause bronchospasm, perhaps by histamine liberation. McIntyre (1947) believes there may also be some central respiratory depression, but Harvey (1940) showed that full curarization by means of quinine methochloride had no effect on the discharge of nerve impulses along the phrenic nerve.

The laryngeal muscles are relaxed, thus facilitating intubation. The cough reflex is not abolished so long as diaphragmatic movements remain.

Curare and Other Systems

Although curare abolishes the nicotinic actions of acetylcholine it has very little or no effect on the muscarinic actions of acetylcholine, so that secretions and smooth muscle reactions are generally little altered.

Urine volume is decreased during moderate or deep curare poisoning, and the action of diuretics is depressed. These effects might be due to increased liberation of anti-diuretic hormone from the neurohypophysis.

Fate of Curare in the Body

Absorption. Absorption from the unbroken skin does not occur, but some absorption from mucous surfaces has been described (see McIntyre, 1947). The gut does not provide a completely impermeable barrier, but the oral lethal dose is about 50 times the subcutaneous lethal dose. Mahfouz (1949) showed that in rats tubocurarine could be absorbed from the small intestine, but not from the stomach.

Blood Levels and Distribution. Mahfouz (1949) has measured blood and cerebrospinal fluid levels after intravenous injection of tubocurarine in man. The resulting small concentrations of tubocurarine were estimated by recording the degree of inhibition of the acetylcholine induced contracture of the frog rectus abdominis muscle. In blood, no tubocurarine was found in the red cells, and the mean plasma levels after injection of 0.2 mg./kg. of tubocurarine were as follows :—

Time after injection	*Plasma level*	*Degree of paralysis*
3 minutes	4 μg./ml.	full paralysis
15 minutes	2.6 μg./ml.	recovery started
30 minutes	1.0 μg./ml.	apparent recovery.

The 3-minute value corresponds to an even distribution of tubocurarine in the plasma volume.

In one case, the cerebrospinal fluid level was 2.5 μg./ml. 33 minutes after injection.

An interesting fact about the distribution of curare has been established. Experiments on dogs by Harroun and Hathaway (1946), and clinical observations, in patients operated on by Cæsarean Section, by Whitaker and Fisher (1945) and by Gray (1947), all seem to show that curare does not pass across the

placenta from the maternal to the fœtal circulation. There were no signs of paralysis in the fœtus at a time when the mother showed full abdominal relaxation. Buller and Young (1949) have shown in rabbits and guinea pigs that maternal blood levels of tubocurarine equivalent to those produced therapeutically in man, do not cause paralysis of the fœtus, though nerve-muscle preparations of the latter are fully sensitive to tubocurarine. Young (1949) has similarly shown that decamethonium iodide does not cross the placental barrier in rabbits or guinea pigs.

Fate and Excretion. Mahfouz (1949) recovered 30–40 per cent of injected tubocurarine from the urine after therapeutic doses in man. Excretion continued for several hours, even though paralysis lasted only half an hour. In mice 60 per cent of tubocurarine was inactivated within 4 hours, and Mahfouz suggests that some destruction may occur in striped muscle. It has been suggested that the liver and kidney play important roles in the detoxication of curare, but Everett (1948) found no difference between normal rats and rabbits, and nephrectomized, hepatectomized animals, with respect to degree or duration of action of tubocurarine. It therefore seems probable that tubocurarine is extensively destroyed in the tissues throughout the body, and a practical implication of this work is that liver or kidney disease is no contraindication to the administration of tubocurarine.

Clinical Uses of Curare

There is now a very extensive literature on the clinical applications of the actions of curare in causing neuromuscular block, and only some of the more recent contributions can be considered here. Brodie's and Waterton's experiments early in the 19th century suggested the use of curare to abolish the violent muscular spasms of tetanus, and treatment firstly of equine, and later of human cases, was reported in the middle of the century. Other convulsive disorders such as epilepsy and rabies were also treated with curare.

Modern applications of the paralysing actions of curare date from the work of Bremer (1929) and that of Hartridge and West (1931). Bremer showed that decerebrate rigidity in cats could be reduced by curare without the production of muscular paralysis. West demonstrated a similar action, firstly in dogs with parathyroid tetany, and later in patients with spastic disorders. He introduced the term "lissive" to describe such a response. It is

important to emphasize that West used varieties of crude curares, only a few of which were lissive, so that this action might have been due, not to d-tubocurarine, but to unknown substances, whose effects might well have been exerted on the central nervous system. In support of this view, West (1937) found that tubocurarine was ineffective in a patient with spastic paraplegia in whom a crude curare preparation relieved muscular spasm.

Curare in Tetanus

Cole (1935) observed the effects of a partially purified curare preparation in three patients with tetanus and concluded, as have others since, that while curare can control the convulsions in this disease it is very doubtful whether it influences the ultimate prognosis. The control of the convulsions of tetanus is probably more easily affected by central nervous depressant drugs such as barbiturates and bromethol; the effects of mephenesin in this disease will be mentioned later.

Curare in Spastic Disorders

West (1932; 1935) described the clinical use of curare preparations in spastic disorders of different origin. In some instances satisfactory relaxation of muscle spasm resulted without the production of paralysis or other toxic side actions. In Parkinsonian rigidity, however, curare appeared to be no more effective than hyoscine. Burman (1940) tried erythroidine as well as curare, and Harvey and Masland (1941) studied the effects of quinine methochloride on spasticity in man; the latter authors concluded that none of these drugs produced objective signs of improvement.

Schlesinger (1946) administered d-tubocurarine in beeswax and arachis oil by intramuscular injection in order to prolong the muscle relaxing action. In some cases relaxation of spasm lasted for many days, much longer than one would expect from the peripheral blocking action alone; the explanation of this effect is not clear. Weed *et al.* (1948) have also used d-tubocurarine in beeswax and oil successfully in patients with tetanus.

Curare in Convulsive Shock Therapy

The violent convulsions produced by drugs such as leptazol and hexazole, as well as those produced by electro-convulsion therapy, have sometimes produced fractures and dislocations.

The use of curare to abolish these violent movements, and thereby to prevent these complications, apparently does not interfere with the therapeutic effectiveness of the above procedures in depressive states and schizophrenia. The use of curare for this purpose was first described by Bennett (1940) in America. In this country curare was first used about the same time by Palmer, but owing to the war his results were not published till 1946 (Palmer, 1946). There have been numerous publications confirming the value of curare in this field. Hobson and Prescott (1947) have described a technique in which d-tubocurarine Cl (0.3 mg./kg.) and thiopentone (0.3g. i.v.) are used to prevent apprehension and traumatic injury, without reducing the efficacy of electroconvulsion therapy.

Curare in Surgical Anæsthesia

Griffith and Johnson (1942) of Montreal were the first to employ curare as an aid to abdominal relaxation in surgical anæsthesia. They administered the preparation, intocostrin, intravenously to twenty-five patients under cyclopropane anæsthesia and noted relaxation of the abdominal muscles in one minute, the effect lasting 10–15 minutes. They also suggested neostigmine as an antidote for overdosage, though they had no occasion to use it. Since 1942 the use of curare in anæsthesia has become widespread and the correspondingly vast literature cannot be dealt with here in any detail (see McIntyre's "Curare" for references).

The main purpose of curare administration as an adjuvant in anæsthesia is to procure increased relaxation of abdominal muscles without the use of deep anæsthesia of central origin. It is well known that first and second plane surgical anæsthesia are not sufficiently deep to abolish reflexes from the upper abdomen, and though it is possible to administer more general anæsthetic and so to reach the third or even fourth plane, the necessary higher anæsthetic concentrations are very liable to depress the bulbar centres controlling respiration and the circulation, and thereby to produce anoxia and signs of shock ; other toxic actions of the anæsthetic drug would also be enhanced. Now curare has no actions on the bulbar centres, nor does it disturb other physiological functions in the doses required to produce peripheral paralysis, provided that anoxia and CO_2 accumulation are prevented ; it must, however, be remembered that to relax the upper

abdominal muscles some intercostal paralysis must be produced. This paralysis can be simply dealt with, if necessary, by controlled respiration. Tubocurarine has no harmful effects upon the circulation except after rapid injection of large doses.

Technique of Administration of d-tubocurarine Cl in Anæsthesia

After the usual premedication with morphine (10 mg.) and atropine (0.65 mg.), most anæsthetists administer thiopentone before the tubocurarine, as recommended by Prescott *et al.* (1946). Gray (1948) has suggested, however, that a preliminary 5 mg. test dose of d-tubocurarine Cl be injected intravenously, and, if the response to this dose is normal, two minutes later a further dose of up to 10 mg. is given followed immediately by 0.5 G. of thiopentone. (preparations of d-tubocurarine Cl must not be mixed in one syringe with thiopentone). Anæsthesia is maintained with 50 per cent N_2O and O_2+thiopentone, and further injections of 5 mg. of d-tubocurarine Cl intravenously may be given as required. If ether is used it must be given in very low concentrations since it greatly potentiates the paralysing action of tubocurarine (Piek and Richards, 1947). Prescott *et al.* (1946) noted that the duration of action of d-tubocurarine Cl was 45–60 minutes after a single intravenous injection of 0.4 mg./kg. body weight (given after thiopentone).

Gray (1948) has reviewed the results of administration of d-tubocurarine to 8,500 patients during a three year period. He makes the following points :—

(i) Clinical impressions suggest that there is less shock after the use of d-tubocurarine Cl with light anæsthesia than with deep anæsthesia alone.

(ii) The duration and severity, but not the frequency, of post-operative vomiting are reduced.

(iii) To prevent the development of post-operative pulmonary atalectasis neostigmine (2.5 mg.) and atropine (0.65 mg.) should be injected intravenously at the end of the operation. (It should be emphasized that the potentiation of vagal inhibition of the heart by this large dose of neostigmine will not be prevented by this small dose of atropine; indeed the latter may actually stimulate the cardio-inhibitory centre, and even larger doses of atropine may produce initial bradycardia before the

peripheral vagal blocking action develops. It is not surprising that occasional deaths, probably due to cardiac arrest, have followed intravenous injection of this mixture.)

(iv) Bronchospasm is extremely rare after d-tubocurarine (1 case in 2,500 administrations). It may be attributable to the light anæsthesia rather than to tubocurarine itself.

(v) There is no evidence that d-tubocurarine is toxic to liver or kidney, or that it causes any disturbance in carbohydrate metabolism.

A final assessment of the value of curare in anæsthesia cannot yet be given, but its use in expert hands has helped greatly in the production of abdominal muscular relaxation in abdominal surgery, and in the immobilization of the chest in thoracic surgery. Provided that gaseous interchange in the lungs is properly controlled by the anæsthetist, there are very few complications to be expected from its administration.

Synthetic Curariform Agents in Anæsthesia

Dimethyl Ether of d-tubocurarine Iodide. Stoelting *et al.* (1948) have reported on the clinical use of this compound in conjunction with N_2O, ether or cyclopropane. An injection of 2–3 mg. produced muscular relaxation lasting 60–90 minutes, with no significant action on blood pressure; there was mild respiratory depression in 9 of 100 cases.

Decamethonium Iodide (C_{10}). The clinical use of decamethonium iodide as a *muscle relaxant in anæsthesia* has been reported by Organe (1949) and by Hewer *et al.* (1949). It was injected intravenously in total doses ranging from 1.5 to 15 mg.; the initial dose was usually 3–5 mg., and subsequently 1–3 mg. were given at intervals as required. Unlike d-tubocurarine it is miscible with thiopentone. After a single dose muscular relaxation commenced after 2–3 minutes and reached a peak within 4–8 minutes; the duration of action was 15–25 minutes so there was little danger of post-operative respiratory depression. Abdominal relaxation could only be produced by doses which caused some respiratory muscular paralysis (cf. action in cats). Recovery was more rapid than with d-tubocurarine, and no cumulative effects followed repeated injections at appropriate intervals. Decamethonium iodide has been used satisfactorily in conjunction with all the commonly employed general anæsthetics. No immediate side

effects were noted by these authors; there was no bronchial or laryngeal spasm; intracutaneous injection of 0.1 per cent decamethonium iodide produced no signs of histamine release such as occur with this strength of d-tubocurarine Cl.

Young (1949) has used decamethonium iodide to produce muscular relaxation for Cæsarean Section, 3 mg. being injected intravenously at least 10 minutes before delivery. The placenta forms a very efficient barrier to the passage of this substance into the fœtus.

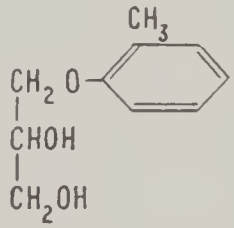
The antagonistic actions of pentamethonium iodide (30–50 mg. intravenously) were not striking and in three cases this substance apparently caused circulatory collapse (Hewer *et al.*, 1949).

Davies and Lewis (1949) and Hobson and Prescott (1949) have used decamethonium iodide in conjunction with thiopentone to prevent the traumatic complications of electrically induced convulsions, and have generally found it preferable to d-tubocurarine.

Flaxedil (*RP* 3697). Mushin *et al.* (1949) have described the clinical use of "Flaxedil" in anæsthesia. They found that 80 mg. of Flaxedil was roughly equivalent in potency to 15 mg. of d-tubocurarine Cl. Wilson and Gordon (1949) have also reported favourably on the use of "Flaxedil."

Mephenesin (Myanesin)

Mephenesin



α:β-dihydroxy-γ-(2-methylphenoxy) propane

Pharmacological interest in this compound relates to its depressant action on the central nervous system. Mephenesin has been used to increase the relaxation of abdominal muscles in anæsthesia, but, unlike curare, it has practically no neuro-muscular blocking action (see reviews by Underhill, 1949, and by Berger, 1949).

The actions of mephenesin were first reported by Berger and Bradley (1946), who studied the effects of a number of simple substituted ethers of glycerol. They selected $\alpha:\beta$-dihydroxy-γ-(2-methylphenoxy) -propane (mephenesin) as the most suitable member of the series for thorough investigation of its action on the central nervous system. Small doses of mephenesin in mice had a tranquillizing effect, and produced muscular relaxation and sleep from which the animals could be roused. Larger doses produced ataxia followed by paralysis, with no reaction to painful stimuli. Recovery from these effects was complete. In rabbits 10–12 mg./kg. of mephenesin (i.v.) produced head drop, and 50 mg./kg. caused complete paralysis, except of the diaphragm. Berger and Bradley (1946) also observed that mephenesin antagonized the convulsant and lethal actions of strychnine more effectively than it antagonized these actions of leptazol, and they therefore suggested that mephenesin acts predominantly on the spinal cord. Mephenesin has a very weak peripheral curare-like action; it is also a local anæsthetic of about the same potency as procaine.

Berger (1947) has studied the actions of mephenesin on the knee jerk in chloralosed cats. Doses of 20–150 mg. somewhat reduced the normal reflex response, but the most striking action of mephenesin was its abolition of strychnine convulsions, with the reduction of the knee jerk to (but not below) its original strength. This selective depression of exaggerated reflex activity, attributed to depression of interneuronal conduction, might be described as a "lissive" action according to the definition of West (1935). It would obviously be much easier to depress augmented reflex activity by an action on the central nervous system than by any influence on neuromuscular conduction; indeed it is difficult to see how a peripheral action could reduce excessive muscle tone without producing paralysis.

In a further study of the actions of mephenesin, Berger (1948) has shown that this compound raises the threshold to electrically induced convulsions in rats, and is actually somewhat more potent than tridione in this respect. Everett and Richards (1949) found that in unanæsthetized cats and rabbits mephenesin, in paralytic doses, produced large slow waves in the electroencephalogram, like those seen after barbiturates. The anticonvulsant activity of compounds of this series is not related to their potency in producing paralysis.

Clinical Uses of Mephenesin

Muscular Relaxation

Mallinson (1947) has recorded the effects of mephenesin in producing muscular relaxation during surgical anæsthesia in 112 patients. Injected by itself into a conscious subject, 27 mg./kg. of mephenesin (i.v.) had no narcotic effect, but produced weakness of limb muscles and full abdominal muscular relaxation. Given in conjunction with thiopentone, 10–15 mg./kg. of mephenesin (10 per cent solution) produced good relaxation of abdominal muscles with very occasional involvement of the intercostals.

Mallinson (1948), at a meeting of the Anæsthetics Section of the Royal Society of Medicine, has reported on some 10,000 administrations of mephenesin chiefly in abdominal surgery, and stated that there were no deaths attributable to this drug. Other anæsthetists at this meeting criticized the use of mephenesin on two main grounds :—

(1) It is less effective than tubocurarine in procuring abdominal muscular relaxation.

(2) It is liable to produce *venous thrombosis* at the site of injection, and *hæmolysis* which may lead to hæmoglobinuria and renal damage.

Damage to veins, and hæmolysis have been reported by many authors, and although the seriousness of these complications has been questioned, they are clearly undesirable (see the review and report of experimental studies by Clendon and Penfold, 1949). The cause of these effects might be mephenesin itself, or the solvent used to make up the 10 per cent solution injected clinically. Mephenesin is only soluble in water at 22°C. to the extent of 1.096 per cent ; it is therefore made up for clinical use dissolved in a mixture of equal parts of ethyl alcohol and propylene glycol. This mixture could undoubtedly damage the vein wall, and also produce hæmolysis. Schlesinger *et al.* (1948) have recommended the use of 2 per cent mephenesin in normal saline to avoid these complications of intravenous injection.

The value and safety of mephenesin as an adjuvant to general anæsthesia still remain to be established.

Hyperkinetic States

Hunter and Waterfall (1948) noted some resemblanees between the aetions of mephenesin and the barbiturates. They were thereby led to test mephenesin in patients with epileptiform fits, and observed that intravenous injection of 0.5 G. abolished fits in three patients within 30 see. They noted too a temporary inhibition of Parkinsonian tremor in one patient after injeetion of 1 G. of mephenesin. Berger and Sehwartz (1948), and Gammon and Churehill (1949) also observed abolition of Parkinsonian tremor, and marked improvement in ehoreoathetosis, after mephenesin. In addition, Gammon and Churehill deseribed abolition of petit mal diseharge by doses of mephenesin whieh did not affect the normal eleetroeneephalogram. These findings, taken with the experimental work of Stephen and Chandy (1947), suggest that mephenesin acts on various parts of the brain as well as on the spinal eord.

Spastic Disorders

Berger and Sehwartz (1948) have administered mephenesin to patients with various spastie and hyperkinetie disorders. They gave the drug dissolved in propylene glyeol by mouth in daily doses of 3–5 G. and in many eases reeorded electromyographie evidenee of improvement. Exaggerated reflexes and rigidity were reduced to normal and voluntary power was retained, the aetion appearing within 20 minutes of administration. Sehlesinger *et al.* (1948) have also reported on the benefieial effects of mephenesin in these eonditions, but they regard the effeets of oral administration as too brief and unpredietable to be of value, and they reeommend intramuseular injeetion of 2 per cent mephenesin in saline.

Tetanus

Benefieial effeets in patients with tetanus have been reeorded after mephenesin by Belfrage (1947), Torren *et al.* (1948), Davison *et al.* (1949), and by Gammon and Churehill (1949).

Other Actions of Mephenesin on the Nervous System

(*i*) *Analgesia.* Gammon and Churehill (1949) observed that mephenesin relieved tabetie and eausalgie pain.

(*ii*) *Action in Psychoses.* Gammon and Churchill (1949) have reported that mephenesin produced temporary beneficial effects in patients with psychoses. They argued that since improvement in such cases results from severance of the connections between thalamus and cortex, a drug which depresses the thalamic region, like mephenesin, might act similarly to produce what one could perhaps describe as a "pharmacological leucotomy." Schlan and Unna (1949) have studied the effects of oral mephenesin in psychiatric patients and found it effective in chronic alcoholism and anxiety states.

Fate and Excretion of Mephenesin

A metabolic product of mephenesin can be detected by the cherry red colour produced with Ehrlich's diazo reagent. It appears in the urine within 15 minutes of oral administration. Two methods for determination of blood and urine mephenesin levels have been described by Titus *et al.* (1948). Inactivation of mephenesin in the body occurs very rapidly (Schlesinger *et al.*, 1948), and it has been shown by Graves *et al.* (1948) and by Riley and Berger (1949), that this is mainly due to oxidation of the terminal CH_2OH group to COOH, the oxidized product, which is pharmacologically inert, being excreted in the urine.

Mephenesin Derivatives and Related Compounds

The brief duration of action of mephenesin is due to the speed of oxidation of the CH_2OH group. Berger and Riley (1949) have found that the acid succinate of mephenesin, in which the CH_2OH group is protected, is less toxic and longer acting than mephenesin itself. This compound forms a very soluble sodium salt which is readily absorbed when given by mouth; it is broken down, probably in the liver, to liberate mephenesin slowly into the bloodstream.

New compounds closely related to mephenesin include some long-acting analogues effective by mouth (Burke *et al.*, 1949), and some 2-substituted-1:3-propane diols which are more powerful anti-convulsants than mephenesin (Berger, 1949). A new compound, 2-methyl, 2-n-amyl-4-hydroxymethyl-1:3-dioxolane ("Glyketal"), has been shown by Berger (1949) to act very similarly to mephenesin.

Other Spinal Cord Depressants

Berger (1949) has recently reviewed the actions of a number of drugs (excluding general anæsthetics) which act predominantly by depressing the spinal cord. Among these are tri-*o*-cresyl phosphate, which produces irreversible paralysis by damaging the anterior horn cells; dithiobiuret, which causes reversible paralysis, but only on prolonged administration; benziminazole and apo-β-erythroidine, which, like mephenesin and glyketal, cause transient paralysis associated with a selective depressant action on interneuronal conduction, and "parpanit" (diethylaminoethyl ester of phenyl-cyclopentane carboxylic acid) which depresses interneurones but does not produce paralysis. Unlike the reflex depression produced by neostigmine (Schweitzer and Wright, 1937) the actions of these compounds appear to be unrelated to transmission of impulses by acetylcholine.

To summarize, it may be said that mephenesin is a central nervous depressant, acting chiefly on the brain stem and spinal cord. It can reduce enhanced excitability to normal and therefore has a truly "lissive" action. Its value as a muscular relaxant in surgical anæsthesia is still doubtful, but its use by mouth in patients with spasticity is well worthy of further investigation. The development of substances which act like mephenesin, but which have more prolonged actions and are more effective by mouth, would be very valuable for the control of the hyperkinetic and spastic conditions discussed above.

Tetraethylammonium Salts

$(C_2H_5)_4\ N^+ ----Br^-$ (or Cl^-)

Quaternary ammonium compounds in general have curare-like actions, but, as mentioned on p. 24, the tetraethyl compound is exceptional in producing no such effect, whereas the tetramethyl compound is very active. Other differences between these two compounds were beautifully analysed by Burn and Dale (1914) who showed in cat experiments that *tetramethylammonium* hydrate produced three types of action, which could be revealed by appropriate procedures :—

(i) A peripheral muscarine-like inhibition of the heart, leading to a fall in blood pressure.

(ii) A nicotine-like stimulation of sympathetic ganglion cells, producing a rise in blood pressure.
(iii) A nicotine-like paralysis of ganglion cells, causing a fall in blood pressure.

Tetraethylammonium hydrate (TEA) had no muscarine, or nicotine-like stimulating actions, but the nicotine-like depression was well shown and could antagonize the ganglionic stimulating effects of tetramethylammonium hydrate.

Acheson and Moe (1946) and Acheson and Pereira (1946) have analysed this ganglionic blocking action of TEA in greater detail, and have shown that the effects of both preganglionic nerve stimulation and acetylcholine injection are prevented by this drug. The depressor action of TEA is entirely attributable to this ganglionic block. Morrison and Farrar (1949) have shown that TEA also prevents the release of adrenaline from the adrenal medulla following splanchnic nerve stimulation in cats.

A new group of *bis*-triethylammonium salts, with ganglionic blocking actions, has been studied by Chou and De Elio (1947). The substances were of the type $(Et_3N(CH_2)_nNEt_3)\ x_2$, where x=the anion (usually Br). Maximal blocking action was seen when n=10, this compound being twice as active as tetraethylammonium bromide on the superior cervical ganglion of the cat. Other ganglionic blocking agents include :—

(1) Pentamethonium iodide, whose actions have already been described.
(2) 2 : 6 dimethyldiethylpiperidinium bromide, whose actions have been reported by Longino *et al.* (1949). It acts like TEA but is 5–7 times more potent.
(3) Lauryl-dimethylamino-2-acetoxy-1-ethane chloride (757L) and lauryl-dimethylamino-3-diacetoxy-1 : 2 propane chloride (758L) (Dallemagne and Philippot, 1949).
(4) Dibutoline (Gruhzit and Moe, 1949).

Tetraethylammonium Salts in Man

Experimental work has shown that the pharmacological actions of the TEA ion are due to its ganglionic blocking effect. The actions on the circulation and on the alimentary tract have been particularly studied in man in relation to tests of autonomic nervous function, and to possible therapeutic applications.

General Effects

Intravenous injection of TEA (Bromide or Chloride) in a dose of 200–500 mg. has been used by Lyons *et al.* (1947), Birchall *et al.* (1947) and Boyd *et al.* (1948). The effects were as follows : within 15–20 sec. of injection there was a metallic taste in the mouth, in 25–35 sec. numbness, coldness and tingling were felt in the hands and feet. Incomplete dilatation of the pupil, cycloplegia and occasionally ptosis were seen, a feeling of tiredness and weakness developed, and sweating ceased. The predominant circulatory effects were a fall in blood pressure within 30–90 sec. of injection, an increase in heart rate to 90–120 beats/min., and a marked rise in skin temperature of the fingers and toes (see also Hoobler *et al.*, 1949). The circulatory effects usually lasted for 15–30 minutes, but postural hypotension might occur for up to one hour. Nearly all these actions may be ascribed to blocking of transmission through the appropriate autonomic ganglia. Doses exceeding 1 G. of tetraethylammonium Cl intravenously may not only produce circulatory collapse, but a curare-like paresis may also develop. Intramuscular injection of 1–2 G. reduced the blood pressure for 2–8 hours. Oral administration was ineffective, owing to poor absorption from the alimentary tract.

On the alimentary tract Holt *et al.* (1947) showed that intravenous injection of 200 mg. of tetraethylammonium Cl diminished the tone and abolished peristaltic activity in the stomach (the same effect as vagotomy). No emptying of the stomach occurred during the period of action of TEA. Macdonald and Smith (1949) also showed that TEA reduced spontaneous gastric secretion and abolished the nervous secretion evoked by insulin hypoglycæmia. Propulsive movements in the small intestine were inhibited and the mucosal markings became fixed. Bladder tone was relaxed. Aas and Blegen (1949) studied the effects of TEAB on renal function in normal subjects. They administered doses (4–6 mg./kg.) which had very little depressor action, and recorded reductions in renal blood flow and glomerular filtration rate, which they attributed to renal vaso-constriction. TEAB also decreased water diuresis and often produced proteinuria. Berry *et al.* (1946) and Conley *et al.* (1947) have recorded relief of causalgia and other types of pain after TEA ; this might have been due to its weak analgesic action described by Hewer and Keele (1948).

Excretion of TEA

Rennick *et al.* (1947) have studied the excretion of TEA in dogs and in man. After intravenous injection in man 50 per cent of the dose appears in the urine within 30 min.; after intramuscular injection 50 per cent excretion occurs within four hours. These authors suggest that TEA should not be given to patients with severe renal disease.

Applications of the Actions of TEA

Peripheral Vascular Disease. It was claimed by Berry *et al.* (1946) that the cutaneous vasodilatation produced by TEA was equal to that which follows procaine injection into the sympathetic pathway, but Boyd *et al.* (1948) found that this response to TEA was irregular, and that the vasodilatation was always less than that produced by paravertebral or local nerve block. Thus TEA is of little value in estimating the probable effects of sympathectomy in patients with peripheral vascular disease, and is certainly of no value as a therapeutic agent in such cases.

Hypertension. TEA reduces the blood pressure in patients with essential hypertension (Berry *et al.*, 1946; Lyons *et al.*, 1947), and this reduction has been used to estimate the probable effects of lumbo-dorsal sympathectomy in this disease. The value of such estimates, whether TEA or sodium amytal be used, has been questioned by Birchall *et al.* (1947), and in any case these methods of producing vasodilatation are of no therapeutic significance. Frew and Rosenheim (1949) have noted that in hypertensive patients the lowest level of diastolic blood pressure produced by injection of tetraethylammonium bromide is the same as that produced by oral administration of sodium propylmethylcarbinylallyl barbiturate (Seconal). Hayward (1948) has reported temporary relief of orthopnœa and dyspnœa and an increase in vital capacity, after the blood pressure has been lowered by tetraethylammonium bromide in patients with hypertensive heart failure, and he suggests that this substance may be useful in the emergency treatment of acute left ventricular heart failure. This beneficial effect might be due to a reduction in venous pressure, as has been reported in heart failure by Relman and Epstein (1949).

Toxic Effects of TEA. Several authors have reported cases in whom TEA has produced temporary marked falls in blood pressure, and Lindgren and Fisk (1948) have observed myocardial

infarction after injection of TEAB in a patient with arteriosclerotic heart disease. Schwartz (1949) has reported the sudden death of a patient with asthma who received 230 mg. of TEAB intravenously, and suggests that asthmatics may be hypersensitive to the drug.

Pentamethonium Iodide in Man

The original observations of Organe *et al.* (1949) on the actions of pentamethonium iodide in man have been confirmed and extended by Arnold and Rosenheim (1949), Arnold *et al.* (1949) and Grob *et al.* (1949). In normal persons it acts very similarly to TEA but it is effective in about 1/10 the dose and, judged by the presence of vasodilatation and postural hypotension, its action lasts for about 1 hour. Intravenous injection of 25–100 mg. of pentamethonium iodide in hypertensive patients produced falls in blood pressure which might last for several hours. An excessive fall in blood pressure was readily counteracted by injection of adrenaline.

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CHAPTER 3

ANTI-HISTAMINE DRUGS

To appreciate the actions of the so-called anti-histamine drugs it is necessary to know the actions of histamine, the effects of which will be briefly discussed.

Histamine

The actions of histamine were first described and analysed by Dale and Laidlaw (1910–11) who studied its effects in various animal species. The general response of smooth muscle towards histamine is contraction, which is most marked in the intestine, uterus and bronchioles. The effects upon the circulation vary according to the species of animal studied; in most species, including man, the blood pressure is reduced, but in rabbits it is initially raised. These variations are related to the differences in response towards histamine of arteriolar muscle, which constricts markedly in rodents, less so in cats, while in dogs and man arteriolar dilatation occurs. Capillaries are always dilated and an increase in their permeability leads to leakage of protein-containing fluid into the surrounding tissue spaces. The effects of histamine on the blood vessels of human skin were very fully described by Lewis (1927). He showed that when 1 in 1,000 histamine acid phosphate solution was pricked into the skin there appeared firstly, a red area 3–4 mm. in diameter, at the site of introduction, later an irregularly outlined red area of 2–3 cm. diameter was seen (the "flare"), and finally there developed a wheal, due to the escape of protein-rich fluid from the capillaries, at the site of the original circumscribed red area. These three effects of histamine are very similar to those which follow the application of a firm stroke to the skin and were summarized by Lewis in the phrase "Triple Response." The components of the "Triple Response" are thus: (i) *Capillary dilatation*, which produces the local red reaction at the site of application of histamine. (ii) *Arteriolar dilatation*, which underlies the spreading flare. This response is mediated by means of an axon reflex, in which histamine provides

the afferent stimulus, and it fails to occur after degeneration of sensory nerve fibres in the injected region. (iii) *Increased capillary permeability* produces the wheal which is precisely confined to the area of the local red reaction. In man, injection of histamine subcutaneously or intravenously produces flushing of the skin of the blush area with a rise in skin temperature, indicating arteriolar, as well as capillary dilatation (Wakim *et al.*, 1949). Dilatation of intracranial vessels, probably in the dura, produces headache.

Injection of histamine into the skin produces itching (Lewis, 1942), but it has been shown by Rothman (1941) that very dilute solutions of histamine (e.g. 1 in 1,000,000) produce whealing without itching. Emmelin and Feldberg (1947) have found that nettle stings contain 0.1 per cent histamine+1 per cent acetylcholine. They showed that while the "nettle rash" was due to histamine alone, the pain was due to a combined effect of histamine +acetylcholine. Thus other varieties of itching might be caused by histamine release in the skin, and pain would result when acetylcholine was also liberated.

Secretory Actions

Histamine is a powerful stimulant to secretion of HCl by the stomach; it is usually stated that histamine does not affect pepsin secretion, but Ashford *et al.* (1949) noted a marked increase in pepsin output in man. It may also stimulate salivary, pancreatic and intestinal secretions but these effects are less marked in man than in some other species of animal.

Histamine is widely distributed in animal tissues where it is held in some bound state in which it is physiologically inactive. Lewis (1927) first showed that it may be readily released from the skin by a variety of injurious physical and chemical stimuli to produce both local effects, such as the triple response, and general effects, such as a fall in blood pressure and increased gastric secretion.

Histamine in Anaphylaxis and Allergy

The part played by histamine in anaphylactic and allergic responses will now be considered. As far back as 1910, in their original description of the actions of histamine, Dale and Laidlaw noted the close resemblance between the effects of this substance and the responses in anaphylactic shock. It was later shown that

the dominant effects both of histamine and of the anaphylactic response were similar in different species of animals; for example, the outstanding feature in guinea pigs is bronchiolar spasm leading to death from asphyxia, in dogs there is circulatory failure due to spasm of the hepatic veins, and in rabbits there is cardio-circulatory failure resulting from contraction of arterioles in the pulmonary bed (Dragstedt, 1941).

The nature of the anaphylactic reaction has been extensively studied as an expression of an antigen-antibody reaction, and the position may be summarized by saying that the characteristic explosive response results from the combination of specific antigen with antibodies attached to fixed tissue cells, and the different symptomatology in different animal species may be related to differences in distribution of the sensitizing antibody in the various organs of the body (Dale, 1948). The demonstration by Best *et al.* (1927) that histamine could be easily extracted from many tissues suggested that this substance might exist in some bound physiologically inactive state, and it thus became possible to regard the actions of Lewis' "H-substance" as due to release of preformed histamine by the action of injurious stimuli. Lewis himself (1927) was the first to formulate clearly the idea that the triple response produced in the skin by local introduction of the appropriate protein in a sensitized person subject to urticaria, was indeed due to the action of "H-substance."

The theory thus postulated that the reaction of specific antigen with cellular antibody caused in some way the release of histamine from its bound inactive state, and it remained only to demonstrate the presence of increased quantities of histamine in tissue or body fluids as the consequence of this antigen-antibody interaction; Dragstedt and Gebauer-Fuelnegg (1932) recorded the presence of a substance in every way resembling histamine in inferior vena caval blood, and in thoracic duct lymph, of dogs subjected to anaphylactic shock, and Bartosch *et al.* (1932) showed that when antigen was added to the fluid perfusing a sensitized guinea pig's lungs, histamine could be detected in the effluent in concentrations sufficient to account for the induced bronchiolar constriction. Schild (1939) used a simple diffusion method to detect histamine released from isolated guinea pig's tissues in anaphylactic shock, and showed that in some organs, e.g. the uterus, the amount of histamine liberated greatly exceeded that required to produce

maximal contraction in that organ, so that the excess would be available for distribution via the blood stream to organs in other parts of the body; the aorta, which released the greatest quantities of histamine, would increase the blood histamine level without itself showing any significant response. Dragstedt and Mead (1936) showed in dogs that the level of histamine in blood or thoracic duct lymph was proportional to the severity of anaphylactic shock, and Code (1939) showed further that in guinea pigs sufficient histamine was liberated during anaphylactic shock to account for the death of the animals.

"Intrinsic" and "Extrinsic" Histamine

Dale (1948) has recently discussed an aspect of histamine release which may be important in relation to the action of anti-histamine drugs. As already mentioned, the histamine released by the antigen-antibody reaction may produce a response either in the cells which release it, or in remote parts of the body. Dale speaks of the former as an *intrinsic* and of the latter as an *extrinsic* histamine action; as examples of *intrinsic* histamine action the anaphylactic responses of smooth muscle such as the uterus, gut or bronchioles may be cited, whereas the vascular responses in the skin and nasal mucosa would serve as examples of *extrinsic* histamine action, since the latter is released from the epidermis or naso-pharyngeal epithelium and diffuses thence to the subjacent vascular plexus. Anti-histamine drugs might be expected to act more readily against *extrinsic* than against *intrinsic* histamine, in the same way that atropine more easily suppresses the actions of injected acetylcholine than those resulting from its release at cholinergic nerve endings. The much greater effectiveness of the anti-histamine drugs in urticaria and vasomotor rhinitis than in spasmodic asthma may be related to this difference in the site of release of histamine.

Whilst it would appear well established that histamine plays an extremely important part in the type of anaphylactic response already described, it does not explain all the manifestations of this state. For example, incoagulability of the blood has long been recognized as a feature of anaphylactic shock in dogs, and this has been shown by Jaques and Waters (1940) to be due to the liberation of heparin into the blood, from which it was isolated as the barium salt. Kellaway and Trethewie (1940) found that, in addition to

histamine, "slow reacting substance" (Feldberg and Kellaway, 1938) appeared in the perfusate after the injection of antigen into the fluid perfusing sensitized guinea pig's lungs, and Katz (1942) also showed that in sensitized human skin the application of ragweed antigen not only liberated histamine but also this "slow-reacting substance." Furthermore, whilst histamine appears quite clearly to be the cause of death in the early stages of the anaphylactic reaction in guinea pigs and dogs, death at later stages may occur with normal blood histamine levels.

The rôle of histamine in other specific hypersensitivity reactions, such as the Arthus phenomenon, serum myocarditis, tuberculin type reactions or contact dermatitis is not so clear, and if it is concerned in the vascular responses of such inflammatory states its release must be slow and continuous to account for the prolonged vasodilatation characteristic of these responses.

Histamine in Human Allergy

Rose (1947) has reviewed the experimental work in this field. Studies on blood histamine levels in allergic states have given conflicting and generally not significant results, which may be related to the fact that allergic phenomena are usually confined to one or two specific tissues, whereas acute anaphylaxis is a very widespread reaction. Rose has shown too that injection of histamine in man in doses adequate to produce symptoms may cause no increase in blood histamine concentration, so the demonstration of systemic effects, such as flushing of the skin, or increased secretion of HCl in the stomach, would appear to be the most satisfactory evidence of histamine release from tissues during allergic reactions. Katz's (1942) demonstration of histamine release by intradermal injection of antigen in allergic patients is the clearest confirmation of Lewis' original hypothesis.

Weiss *et al.* (1932) were the first to show that whilst histamine injection caused no respiratory symptoms in normal persons, in asthmatic subjects it produced difficulty in breathing with marked reduction in vital capacity. This response has also been recently studied by Curry (1946). It has sometimes been suggested that such findings demonstrate an increased sensitivity of the bronchiolar muscle towards histamine in asthma, but this does not necessarily follow. It may well be that in asthmatics there is some swelling of the respiratory tract mucosa, so that the lumen

is reduced in diameter, though not sufficiently to produce symptoms at rest. Injection of histamine might then so narrow the bronchiolar lumen as to produce symptoms and reduction in vital capacity, even though the increase in muscular tone were no greater than in normal persons.

Anti-histamine Drugs

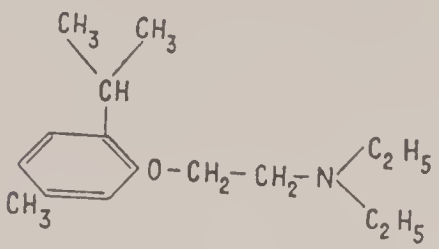
Drugs which antagonize the effects of histamine in animals might work in three main ways. Firstly, they might exert pharmacological actions directly opposed to those of histamine, as for example when adrenaline relaxes the smooth muscle contraction produced by histamine in the intestine or bronchioles, secondly, they might merely prevent or oppose the actions of histamine without themselves having direct antagonistic pharmacological actions, or thirdly, they might destroy or neutralize histamine by some direct chemical action. It is the second type of action [which might in some instances be due to competitive antagonism (see p. 335)] with which we shall be chiefly concerned, since many substances have recently been discovered which are very powerful histamine antagonists of this kind.

Histamine can actually desensitize tissues to its own action, and Edlbacher *et al.* (1937) found that certain amino-acids, e.g. histidine, arginine and cystine, antagonized histamine when applied in very high concentrations, but the discovery of the really potent anti-histamine drugs originated with the work of Bovet and his colleagues in the Pasteur Institute in Paris. (See reviews on anti-histamine drugs by Feinberg, 1946 and 1947; Loew, 1947; Gaddum, 1948; Dale, 1948; Bovet and Bovet-Nitti, 1948.)

Ungar *et al.* (1937) showed that an anti-adrenaline drug, piperidinomethyl-2-benzodioxane (933F) also antagonized histamine, and Bovet and Staub (1937) found that a phenolic ether, 929F, had definite anti-histamine actions, although too toxic for therapeutic use. Modifications of its structure led to more active compounds and the relations of some of these substances to 929F are seen in the chemical formulæ. In 1939 Staub introduced the first ethylenediamine derivative (1571F), and in 1942 Halpern described his observations on two compounds prepared by Mosnier, 2325 R.P. and 2339 R.P. (Antergan), the latter being a much more powerful histamine antagonist than 1571F. In 1944, Bovet

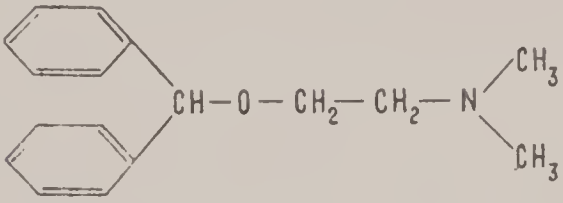
Chemical Formulæ of Some Anti-histamine Drugs

929F



2-isopropyl-5-methylphenoxyethyldiethylamine

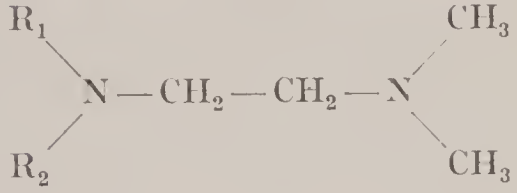
Benadryl (Diphenhydramine)

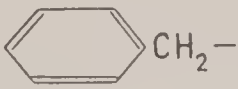
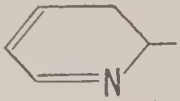
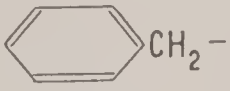
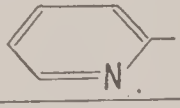
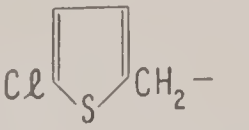
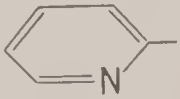


β dimethylaminoethylbenzhydryl ether

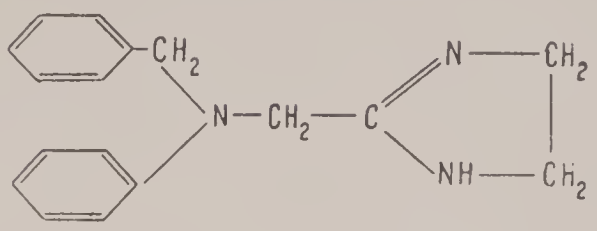
Derivatives of Ethylenediamine

General formula



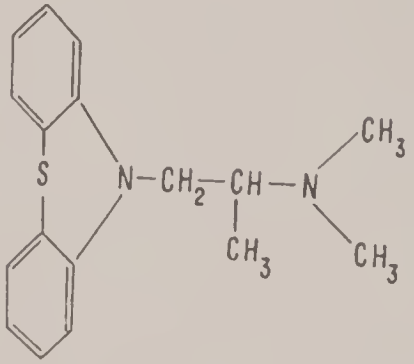
	R1	R2
Antergan (2339 R.P.)	 (Benzyl)	(Phenyl)
Mepyramine (2786 R.P., Neoantergan, Anthisan)	(p-methoxybenzyl)	 (2-pyridyl)
Pyribenzamine (Tripelennamine)		
Chlorothen		

Antistin



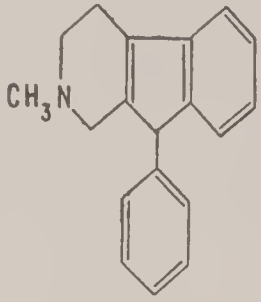
2-(N-phenyl-N-benzylaminomethyl)-iminazoline

Promethazine
(Phenergan
3277 R.P.)

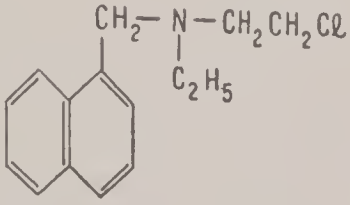


β-dimethylamino-N-propylthiodiphenylamine

Thephorin
(Phenindamine)



2-methyl-9-phenyl-2, 3, 4, 9-tetrahydro-1-pyridindene



N-(2-chloroethyl)-N-ethyl-1-naphthalenemethylamine

et al. introduced the still more potent Neoantergan (Mepyramine), and in 1945 Mayer *et al.* described the closely related compound Pyribenzamine. In 1945, Loew *et al.* introduced Benadryl (Diphenhydramine) and in 1947 Halpern reported the powerful anti-histamine actions of Promethazine (3277 R.P., Phenergan). Other drugs of this group include Antistin (Meier and Bucher, 1946), Hetramine (Feinstone *et al.*, 1946), Neohetramine (Reinard and Scudi, 1947), Chlorothen (Litchfield *et al.*, 1947) and Thephorin (Lehmann, 1948), which is very interesting since its chemical structure is quite different from the others.

Loew and Micetich (1948) and Stone and Loew (1948) have recently described a series of compounds which, unlike most anti-histamines, antagonize the effects of adrenaline as well as those of histamine. The most important member of this series is N-(2-chloroethyl)-N-ethyl-1-naphthalenemethylamine, which is as potent as mepyramine in the protection of guinea pigs against histamine toxicity or anaphylactic shock.

Actions of Anti-histamine Drugs

All the actions of histamine in the body, except the stimulation of HCl secretion by the stomach, are antagonized by the anti-histamine drugs. The simplest demonstration of this antagonism is the prevention of death from histamine poisoning by prior administration of an anti-histamine drug. This has been the basis for many studies on the relative potencies of drugs of this group and has been most simply performed on guinea pigs, in which histamine, usually given intravenously or in atomized form by inhalation, causes asphyxial death in a few minutes from bronchiolar spasm and peribronchial and mucosal œdema. For example, Friedlaender *et al.* (1947) determined the number of L.D.100 doses of histamine required to kill guinea pigs after intraperitoneal injection of some anti-histamine drugs, each given in a dose of 3 mg./kg. 15 minutes before intravenous injection of histamine. They obtained the following values :—

After Benadryl 5 L.D.100 doses of histamine were required to produce 100 per cent of deaths.

After Antergan 6 L.D.100 doses of histamine were required to produce 100 per cent of deaths.

After Pyribenzamine 37 L.D.100 doses of histamine were required to produce 100 per cent of deaths.

After Mepyramine 125 L.D.100 doses of histamine were required to produce 100 per cent of deaths.

Halpern (1947) found by this method that promethazine could protect guinea pigs against up to 1500 L.D.100 doses of histamine, a remarkably powerful antagonism.

Comparative tests of this kind may exaggerate the differences between individual drugs, but there is no doubt that pyribenzamine, mepyramine and promethazine are more effective than antergan, benadryl, and antistin in the prevention of histamine induced broncho-spasm, and the former are also generally more potent against clinical allergic diseases.

Anti-histamine drugs can prevent the blood pressure lowering action of histamine in anæsthetized cats or dogs. They also antagonize the actions of histamine on the smooth muscle of isolated organ preparations such as the intestine, uterus and perfused lung. The histamine-induced contraction of the guinea pig ileum is very suitable for comparative studies on anti-histamines, since the histamine stimulus may be applied repeatedly at short intervals (e.g. 3 minutes) and the anti-histamine drug actions are usually quite easily removed by washing out. The quantitative aspects of this technique have been very fully investigated by Schild (1947) who found mepyramine to be the most potent anti-histamine substance on the guinea pig ileum. Reuse (1948) has used mepyramine, in low concentrations, as a means of identifying histamine in tissue extracts, in the same way that atropine can help in the identification of acetylcholine.

Capillary Permeability. Histamine antagonists can prevent the increase in capillary permeability produced by histamine (Last and Loew, 1947). Both benadryl and mepyramine, given subcutaneously in rabbits, could diminish or prevent the escape of intravenously injected trypan blue at sites of intradermal injection of histamine. Halpern (1949) has particularly emphasized that promethazine opposes the increase in capillary permeability evoked by other substances besides histamine. He found that it could prevent the appearance of the acute pulmonary œdema induced by adrenaline, chloropicrin or phosgene; promethazine also prevented the production of experimental orthostatic albuminuria

in rabbits. Halpern has, therefore, suggested that apart from its anti-histamine action, promethazine has some direct effect on capillary permeability. This action might be related to the finding of Haley and Harris (1949) that promethazine, and some other histamine antagonists, cause powerful contraction of the pre-capillary sphincters in the vessels of the rat's meso-appendix. The concentration of promethazine required for this action was of the order to be expected in tissues from administration of the usual doses of the drug by mouth or by injection. Last and Loew (1947) showed that the increased permeability produced by intradermal injection of trypsin, snake venom, staphylococcus toxin or other noxious substances, as well as that resulting from injection of horse serum into sensitized rabbits to produce the Arthus reaction, were not modified by benadryl. However, Benacerraf and Fischel (1949) have found that very large doses of promethazine (25–100 mg./kg.) can inhibit severe Arthus reactions, probably by the vascular mechanisms just described. This drug also inhibited the skin responses to streptococcal erythrogenic toxin. It is still not clear what part, if any, is played by histamine in such reactions.

Macht *et al.* (1949) have noted that benadryl and pyribenzamine (introduced by iontophoresis) inhibited the formation of experimental frost bite wheals in human skin. The drugs also reduced the tendency to vesicle formation, but had no effect on the tenderness, pain and redness which were present for the first few days after freezing.

Inhibition of Histamine Wheal by Anti-histamine Drugs in Man. Bain and his colleagues at Leeds (Bain *et al.*, 1948, and Bain, 1949) have studied the actions of anti-histamine drugs, given both locally and by mouth, on the development of the flare and wheal responses to intradermal injection of histamine in normal human subjects. By measuring the amount of drug required to reduce the area of histamine-induced wheals by 50 per cent they were able to compare the *weight for weight potencies* of anti-histamine drugs. For example, they found after oral administration, the following equivalent potencies for single doses of the drugs mentioned: 40 mg. promethazine ≡ 275 mg. mepyramine ≡ 600 mg. antistin. In other words promethazine was nearly seven times as potent as mepyramine, and fifteen times as potent as antistin. Bain *et al.* also used this inhibition of histamine wheal formation to follow the

duration of action of anti-histamine drugs. The results will be discussed later.

Anti-anaphylactic Actions. As was to be expeeted, anti-histamine drugs ean prevent the symptoms and signs of anaphylactie shoek in animals. This effect has been most simply demonstrated in

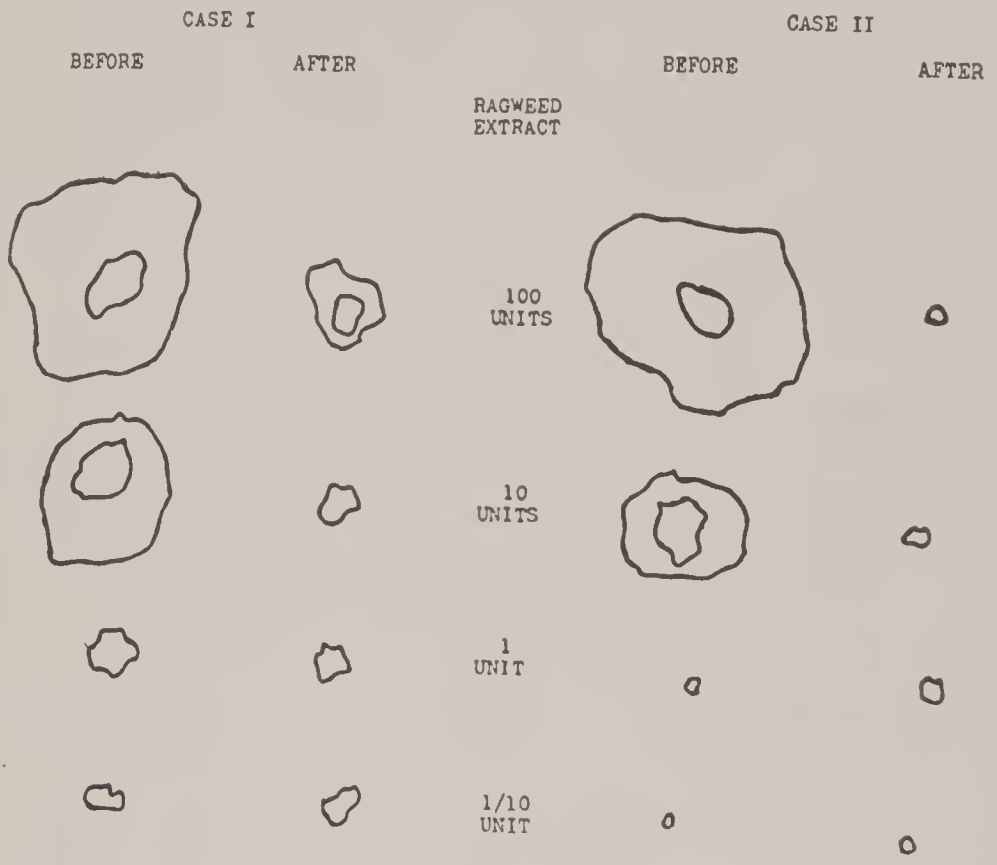


FIG. 6

The responses to ragweed extract were tested in 2 allergic subjeets. 45 minutes after oral administration of 100 mg. of pyribenzamine the flare and wheal responses were much smaller than in the control observations.

(After Arbesman *et al.*, 1946.)

guinea pigs, and it is interesting to note that the differences in poteney among drugs of this group are mueh less with respect to their anti-anaphylactie, than to their anti-histamine aetions. For example, Friedlaender *et al.* (1946) found that although pyribenzamine was seven times more powerful than benadryl against histamine shoek, there was no difference in their capaeity to

prevent the anaphylactic response. Landau *et al.* (1948) showed that in spite of wide differences in anti-histamine potency, benadryl, pyribenzamine, mepyramine, chlorothen and promethazine were equi-active against anaphylactic shock in guinea pigs. Antistin was definitely weaker than the other compounds, both against histamine toxicity and anaphylactic shock. Halpern (1947) found that these drugs (e.g. promethazine) could not only prevent the acute shock but also the tissue eosinophilia as seen, for example, in the lungs.

In man, Harley (1946) showed that benadryl reduced the skin response to grass pollen extracts in allergic subjects, and Arbesman *et al.* (1946) have shown a similar effect with pyribenzamine (Fig. 6). Herxheimer (1949) has also demonstrated the prevention of mild pollen-induced bronchospasm by means of mepyramine and promethazine.

The possibility that anti-histamine drugs might prevent the development of chronic allergic tissue reactions has been studied, among others, by MacGregor and Wood (1949). They injected horse serum into rabbits and noted the previously reported appearance of myocardial and valvular lesions resembling those of rheumatic fever or polyarteritis nodosa. Mepyramine and benadryl had no effect on the development of these lesions.

Gastric Secretion. It is a very interesting finding that anti-histamine drugs do not inhibit the secretion of HCl evoked by histamine (Loew, 1947). Indeed there is some evidence that these drugs may actually increase the gastric secretory response to histamine in cats and in man (Wood, 1948; Doran, 1947; Ashford *et al.*, 1949). A most striking demonstration of this anomalous finding is seen in the work of Halpern (1947) who showed that although guinea pigs could be protected against the immediate effects of up to 1500 lethal doses of histamine by promethazine, many animals died 24–48 hours later with acute perforations of the stomach, presumably due to the very powerful stimulation of HCl secretion by the histamine.

Anti-histamine drugs have no effect on normal gastric secretion.

Other Actions of Anti-histamine Drugs. Apart from their antagonism towards histamine these drugs have certain other actions.

(*i*) *Local Anæsthesia.* All the anti-histamine drugs are local anæsthetics. For example Dews and Graham (1946) found that

mepyramine was about three times as powerful as procaine. It would seem probable that anti-histamine action is not related to local anæsthetic potency.

(*ii*) *Quinidine-like Action.* Dews and Graham (1946) found that mepyramine was twice as potent as quinidine in prolonging the refractory period of the rabbit's auricle.

(*iii*) *Anti-acetylcholine Action.* These drugs produce atropine-like effects, e.g. dryness of the mouth. They antagonize the actions of acetylcholine on smooth muscle but Schild (1947) has shown that higher concentrations are required for this effect than to antagonize the actions of histamine. Mepyramine in particular is highly specific in its antagonism to histamine, its anti-acetylcholine action being very weak.

(*iv*) *Anti-adrenaline Action.* A few members of this group antagonize certain actions of adrenaline, e.g. N-(2-chloroethyl)-N-ethyl-1-naphthalenemethylamine (Loew and Micetich, 1948) and thephorin (Lehmann *et al.*, 1949).

(*v*) *Central Nervous System.* In man these drugs, especially benadryl, frequently produce drowsiness. Hewer and Keele (1948) have shown that benadryl and mepyramine have analgesic actions in man. These central effects may explain the relief produced in some patients with pruritus.

Churchill and Gammon (1949) have studied the actions of benadryl and pyribenzamine in patients with epilepsy. Benadryl, acting like mephenesin, diminished the electroencephalographic abnormality, and reduced the frequency of attacks in petit mal, but actually induced seizures in patients with focal lesions of the cerebral cortex. Pyribenzamine increased the abnormality in both petit mal and focal cases.

(*vi*) *Action on Hyaluronidase* (*spreading factor*). It has been claimed by Mayer and Kull (1947) and by Elster *et al.* (1949) that *in vivo* pyribenzamine antagonizes the spreading action of hyaluronidase. Since there is no such antagonism *in vitro* it is possible that this effect is due to some compound formed from pyribenzamine in the body.

Absorption and Excretion of Anti-histamine Drugs

McGavack *et al.* (1948) followed the *absorption* of benadryl and pyribenzamine by determining blood concentrations after oral administration of 400 mg. of each drug. With benadryl, the

blood level began to rise about one hour after ingestion, and reached a peak value of about 1 μg./ml. after 1½–2 hours; with pyribenzamine the blood levels rose more slowly and had not reached a peak after three hours.

Both drugs were *excreted* in the urine. After 24 hours 46 per cent of benadryl and 20 per cent of pyribenzamine were recovered. However, it seems highly probable from the work of Glazko *et al.* (1949), who studied the distribution and excretion of benadryl containing radioactive carbon, that about 90 per cent of the material present in the urine represents degradation products of benadryl. Glazko and Dill (1949) incubated benadryl with various tissues in rats, guinea pigs and rabbits and found that liver caused the greatest destruction of the compound, though lung and spleen were also active.

Perlman (1949) has described a method for the determination in body fluids of anti-histamines which contain pyridine and dimethylethylenediamine groupings (e.g. pyribenzamine and mepyramine). These substances react with cyanogen bromide to produce a blue fluorescent substance. After oral administration of pyribenzamine the highest rate of excretion occurred within 1½–4½ hours, and the maximum amount excreted was about 10 per cent of that ingested. The excreted pyribenzamine was firmly combined with some acidic substance or radical.

Clinical Uses of Anti-histamine Drugs

It is not possible in the available space to review the whole of the extensive literature in this field, but there are a number of general aspects and certain conclusions to be discussed. The reviews by Feinberg (1946, 1947), Hunter and Dunlop (1948), Loew (1947) and Loveless and Dworin (1949) should be consulted.

In the first place it must be emphasized that the anti-histamine drugs are purely palliative in their actions. They have no effect on the development of sensitization, and they do not interfere with the presumed antigen-antibody reactions which underlie the production of allergic manifestations. They are in no way substitutes for desensitization procedures, nor do they replace such adjuvant drugs as spasmolytics, vasoconstrictors or expectorants. Moreover they do not influence appreciably the "delayed" types of inflammatory allergic reaction, and are not even uniformly effective against the "immediate" types. Gay *et al.*

(1948) have emphasized that the clinical effectiveness of the various anti-histamine drugs is much more closely related to their anti-anaphylactic than to their anti-histamine potencies in animals.

Effects in Urticaria, Serum Sickness and Dermographism

There is no question about the considerable symptomatic value of these drugs, which will first be considered in relation to such characteristic allergic conditions as urticaria, serum sickness and dermographism.

It has been the general experience that about 80 per cent of patients with *urticaria* and *angioneurotic œdema* obtain worthwhile relief from anti-histamine drugs. The relief of itching may be more striking than the diminution of œdema. In serum sickness or in penicillin reactions the joint symptoms are more resistant than the manifestations in skin and mucous membranes. Acute urticaria usually responds better than the chronic forms. Though the drugs prevent the development of new lesions they do not affect established lesions.

According to Feinberg (1947), benadryl and pyribenzamine are about equally effective against urticaria. Bain *et al.* (1948 and 1949) have correlated clinical improvement in urticaria following mepyramine and promethazine, with coincidental reductions in skin responses to histamine. They found that chronic urticaria was just controlled by doses of mepyramine and promethazine which reduced the area of the intradermal histamine wheal by about 50 per cent. They also compared the therapeutic potency of mepyramine and promethazine by finding the smallest dose of each drug which would just prevent the appearance of urticarial lesions ; in this way they showed that 25 mg. daily of promethazine was as effective as 350 mg. daily of mepyramine, or in other words promethazine was 14 times as potent as mepyramine. Against urticaria, antistin appears to be less effective than the other drugs (Britton, 1947).

The palliative, non-curative nature of anti-histamine drug action was well shown by Hunter (1947) who noted recurrence in patients with chronic urticaria when tablets of mepyramine were replaced by dummy tablets. There is no evidence that the course of acute conditions like serum sickness or penicillin urticaria is shortened by these drugs.

The beneficial action of anti-histamines in dermographism has

been applied by Feinberg (1947) to the suppression of non-specific reactions in the course of skin sensitivity tests in allergic subjects.

In cases of chronic urticaria in which mepyramine was given for periods up to two years there was no evidence of any increased tolerance to the drug (Hunter and Dunlop, 1948).

Hay Fever and Perennial Allergic Rhinitis

The general response to anti-histamine drugs in these conditions has been that about 80 per cent of hay fever patients, and about 50 per cent of patients with perennial vasomotor rhinitis, show definite improvement. Pyribenzamine and mepyramine appear to be more effective than benadryl or antistin. In allergic rhinitis the most striking effect is the relief of itching and sneezing. Reduction of intranasal œdema is less marked, and it may be desirable in some cases to add the vasoconstricting properties of ephedrine to the effects of the histamine antagonist.

It will be recalled that in urticaria and hay fever we are dealing with the actions of what Dale (1948) has called "extrinsic" histamine. This may account for the good responses obtained with anti-histamine drugs.

Asthma

Although earlier clinical studies suggested that anti-histamines produced improvement in asthma, it is now generally accepted that these drugs are of little or no benefit in this condition, except perhaps in the mild chronic asthmatic state (Herxheimer, 1949).

This has been most clearly revealed by the investigations of Levy and Seabury (1947) who observed no increase in vital capacity in asthmatics after benadryl, and by the carefully controlled clinical studies with mepyramine by Hunter and Dunlop (1948) and Southwell (1948). The reasons for this poor response are not clear. Curry (1946) measured the reduction in vital capacity produced by injection of histamine in asthmatic subjects and showed that benadryl or pyribenzamine could completely prevent this action of histamine (Fig. 7). However it will be noted that in these experiments of Curry's the histamine is to be regarded as "extrinsic," whereas the allergic response might release histamine of the "intrinsic" type in very close relation to the bronchiolar muscle and mucosa. The concentration of

anti-histamine drugs might be adequate to counteract "extrinsic" but not "intrinsic" histamine, and to overcome this difficulty Feinberg has suggested the inhalation of aerosols of anti-histamine drugs.

However it still remains possible that asthma is not primarily due to the actions of histamine at all. It may be mentioned that

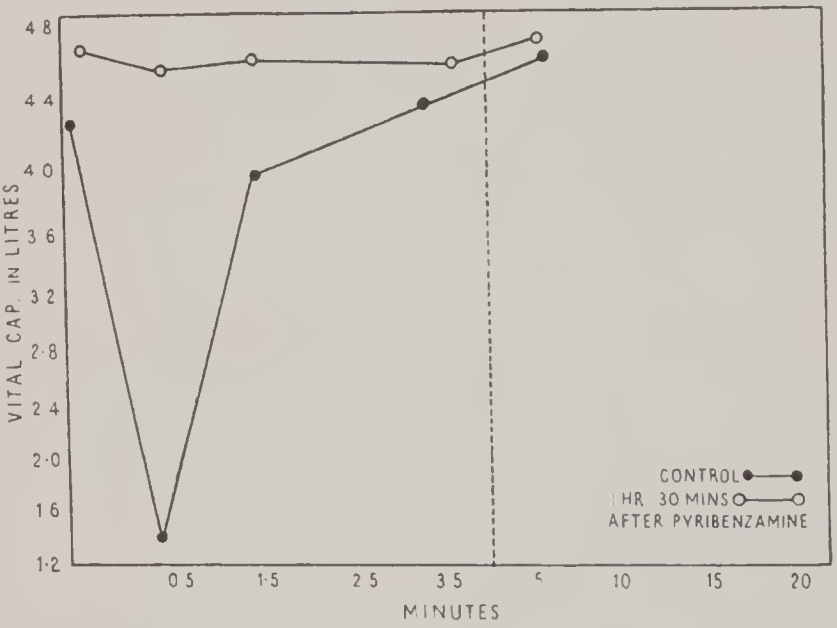


FIG. 7

PYRIBENZAMINE ON HISTAMINE BRONCHOCONSTRICTION

Intravenous injection of 0.02 mg. of histamine caused marked temporary reduction in vital capacity due to bronchoconstriction. Previous administration of 50 mg. of Pyribenzamine by mouth prevented this action of histamine.

(After Curry, 1946.)

Curry (1947) found that asthmatic subjects were even more sensitive to the actions of acetyl-β-methyl choline than to histamine.

Skin Diseases Other than Urticaria

The itching of atopic dermatitis usually responds well to anti-histamine drugs, and the subsequent cessation of scratching may allow the skin condition to improve. In contact dermatitis,

eczema and some types of pruritus, itching may also be relieved, though usually less effectively than in atopic dermatitis. Anti-histamine drugs have usually been administered orally in these conditions, but Feinberg and Bernstein (1947) have reported good results with an ointment containing 2 per cent pyribenzamine hydrochloride.

Strauss (1949) has reported very rapid relief of pain and local manifestations after application of 5 per cent thephorin ointment to sites of bee stings. It was also effective against stings by wasps and hornets, and against bites by ants and mosquitoes.

Miscellaneous Uses

Anti-histamine drugs have been described by some as effective in gastro-intestinal allergy, in migraine, in erythema multiforme, in allergic eye diseases and in drug eruptions of the urticarial type.

The anti-histamine drugs have been found useful in the control of local and systemic reactions produced by specific antigens during desensitization therapy. They can also be used to prevent local hypersensitivity reactions to injection of liver extract or insulin (e.g. Hunter and Hill, 1947).

It has been claimed by Craig *et al.* (1949) that mepyramine is effective in acute nephritis. If this report is confirmed it would be most interesting, since it is difficult to see how the effect could be due to the anti-histamine properties of the drug, and it might be that mepyramine in some way influences the allergic process thought to underlie this condition.

It has been stated by Brewster (1949) that a number of anti-histamine drugs were effective in the treatment of the common cold, but a properly controlled study by Paton *et al.* (1949) on the actions of mepyramine in this condition did not confirm this claim.

Central Nervous Actions of Anti-histamines

It is now well recognized that anti-histamines exert definite effects on the central nervous system, and reference has already been made to the soporific and analgesic actions of these drugs. It seems likely that certain therapeutic applications of these compounds are based on their central nervous actions.

Parkinsonism. Beneficial effects from anti-histamine drugs in

Parkinsonism have been reported in France and the U.S.A., and Ryan and Wood (1949) have described their results of administration of benadryl to 40 patients with this disease. The most striking benefit was relaxation of rigidity, tremor usually showing no improvement.

Actions on the Vomiting Centre. Bignall and Crofton (1949) have described the successful use of benadryl and antistin in the relief of nausea and vomiting produced during treatment with streptomycin. These authors emphasize that the anti-histamine drugs have no effect on the giddiness due to streptomycin. However, Brown and Hunter (1948) observed no benefit from administration of mepyramine to patients with irradiation sickness ; indeed there was more constitutional upset in treated patients than in untreated controls.

A most interesting, and useful, application of an anti-histamine drug is described by Gay and Carliner (1949). They gave "dramamine" (β-dimethylaminoethylbenzohydryl-ether-8-chloro-theophylline) to a pregnant woman with urticaria, who noted that the drug completely prevented car sickness, to which she was very predisposed. The authors then organized a large scale trial of the drug for its effects on seasickness in 485 American soldiers travelling in a liner from U.S.A. to Bremerhaven. Carefully controlled observations showed that "dramamine" was much more effective than a placebo, both in the prevention and treatment of seasickness. For example, 372 of 389 seasick soldiers were completely relieved within one hour of oral administration of a single 100 mg. dose of "dramamine"; the usual dosage was 100 mg. five hourly, which produced no side effects. Rust and Fosbery (1949) have confirmed these findings, and have found "dramamine" to be effective in persons who do not respond to hyoscine. They regard 50 mg. twice daily as an adequate dose. Strickland and Hahn (1949) also found that "dramamine" was useful in the prevention of airsickness, but the effects were less striking than those reported in seasickness. Carliner *et al.* (1949) have also reported beneficial effects from administration of "dramamine" to women suffering from vomiting of pregnancy.

McEvedy (1949) has compared the effects of mepyramine and hyoscine in the control of seasickness ; he found 100 mg. of mepyramine at least as effective as 0.65 mg. of hyoscine, and recommended three daily doses of the former drug.

Administration and Dosage

The anti-histamine drugs are usually administered orally in tablet, capsule or liquid form (see Hunter and Dunlop, 1948). They may occasionally be given by subcutaneous, intramuscular or slow intravenous injection. The usual single oral dose of benadryl and pyribenzamine is 50 mg., and of mepyramine and antistin 100 mg. The maximum daily dose of benadryl is about 400 mg., of pyribenzamine 600 mg. and of antistin and mepyramine 800 mg. As Bain *et al.* (1948) have shown, even larger doses of mepyramine (up to 1,200 mg. daily) may be required to control urticaria in some patients, but of course side actions are much more frequent with such large amounts.

The drugs are generally fairly rapidly absorbed from the alimentary tract and peak action occurs after 1½–4 hours. It is usually stated that the actions persist for 4–6 hours, but Bain *et al.* (1948 and 1949) have shown that promethazine differs markedly from the other anti-histamine drugs in having a very prolonged action. For example, they studied the duration of action of some of these drugs by recording their inhibitory effects on the size of the intradermal histamine wheal, induced at frequent intervals. To compare the drugs with each other they measured the time of decay from full action to half action and obtained the following figures (see Fig. 8).

Promethazine	19½ hours
Mepyramine	5 hours 10 minutes
Antistin	3½ hours
Pyribenzamine	3¼ hours

Perry and Hearin (1949) have recorded the duration of action of anti-histamine drugs by measuring the cutaneous response to different concentrations of histamine introduced by iontophoresis. With 50 mg. of benadryl or pyribenzamine, the maximum effect occurred about 2 hours after ingestion, and the action was over in 5 hours.

Bain *et al.* have taken advantage of the prolonged action of promethazine in the following way. A single dose of 25–50 mg. is given daily at night. The side effects are insignificant during the night (indeed the soporific action may be helpful) and pass off before the morning, whilst the therapeutic effect is much more prolonged,

lasting for over 24 hours. Administration of the other anti-histamine drugs is usually required three times daily, but the spacing of dosage should be related to the time of day when allergic symptoms are to be expected.

Toxic Effects of Anti-histamine Drugs

Toxic side effects with these drugs are fairly frequent (20–50 per cent of cases). Mepyramine and pyribenzamine are better tolerated

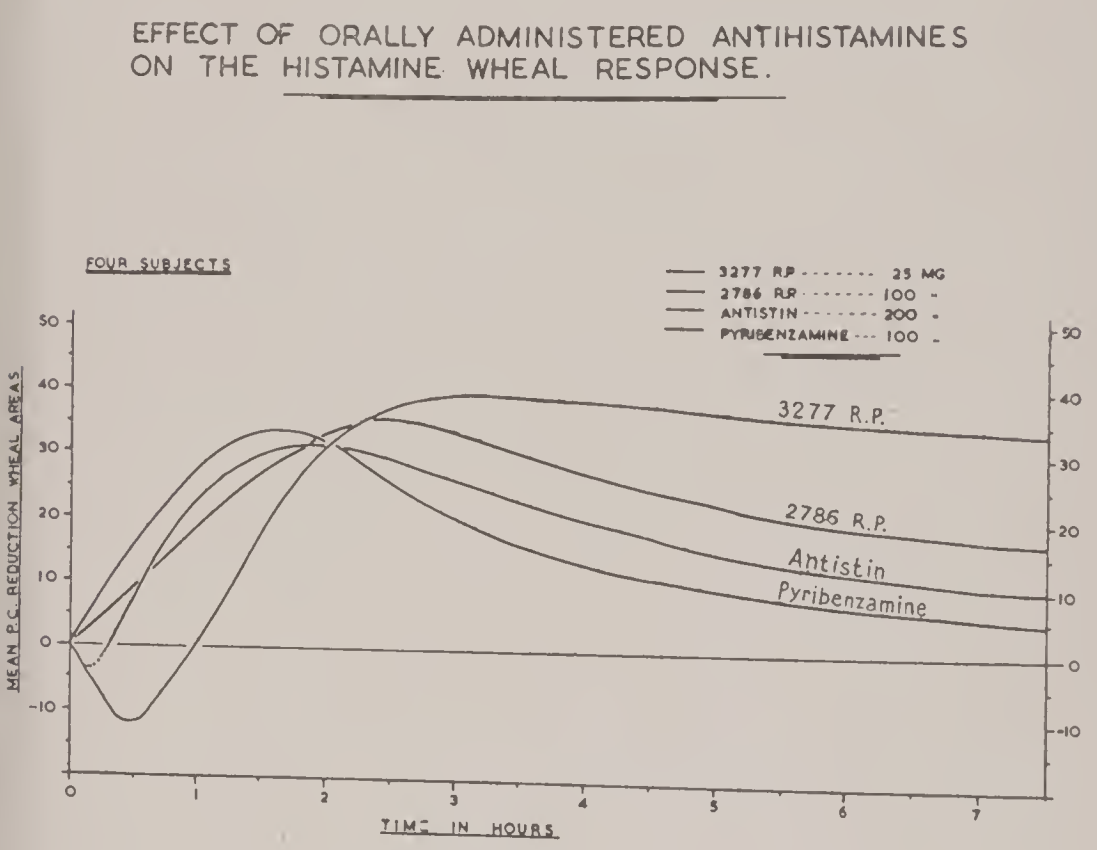


FIG. 8

DURATION OF ACTION OF ANTI-HISTAMINE DRUGS IN MAN

The actions of the various drugs were estimated at intervals by measuring the area of the wheals produced by intradermal histamine.

3277 R.P. = Promethazine (Phenergan)
2786 R.P. = Mepyramine (Anthisan)

Note the very slow rate of decay in the action of promethazine.

(After Bain, 1949.)

than benadryl and, since they are also more potent, they are therapeutically more effective. The commonest symptoms are drowsiness and lassitude which are most frequently seen with benadryl, and least with thephorin (Frank, 1948), which actually more frequently produces insomnia and other signs of central nervous stimulation. Such symptoms are commoner in ambulant patients than in those resting in bed. Amphetamine may be given to overcome the soporific action of benadryl. Other undesirable actions are dizziness, headache and dryness of the mouth and nose. Nausea, vomiting, mild colic and diarrhœa (most frequent with antistin, mepyramine and pyribenzamine) can usually be avoided by taking the drugs in the middle of or after meals. Promethazine produces lightheadedness, aching limbs and ataxia (Bain *et al.*, 1948).

An interesting complication following local application of pyribenzamine has been the development of dermatitis (Epstein, 1947). The prolonged local use of thephorin in chronic pruritic dermatitis led to final exacerbation of the condition in 14 of 50 patients studied by Ellis and Bundick (1949). In 5 of the 14 patients patch tests were done ; all were positive. It is certainly desirable to know whether prolonged local application (e.g. for 6 weeks or more) of other anti-histamine drugs produces similar reactions.

It is important to emphasize that there are considerable individual variations in the development of these side actions, and if one drug is not tolerated another may sometimes be satisfactory.

It is not yet known whether long periods of administration of anti-histamine drugs have any harmful effects, but so far there has been no evidence of toxic actions on kidney, liver or blood, except for one case of granulocytopenia following pyribenzamine (Blanton and Owens, 1947).

Deaths following accidental overdosage of an anti-histamine drug have been reported. Tobias (1949) has described the case of a child of 21 months who swallowed 600 mg. of mepyramine maleate, and Rives *et al.* (1949) have recorded the case of a child of 16 months who took 100 mg. of Methapyrilene (Thenylene). In both cases, after signs of initial central nervous depression, there were convulsions. Rives *et al.* observed cerebral œdema and upper nephron nephrosis at necropsy.

Conclusions

This discussion of the anti-histamine drugs has been deliberately restricted to those substances whose actions have been sufficiently tested, both experimentally and therapeutically, to justify at least an interim evaluation. The efficacy of these drugs in urticaria and allergic rhinitis seems to be proved, and their relative ineffectiveness in asthma is now generally accepted. It is still difficult to summarize the comparative effectiveness of these drugs. Benadryl is undoubtedly more liable to produce drowsiness than the others ; of the drugs in general use, antistin is definitely the weakest and also the least toxic, weight for weight, and promethazine is clearly the longest acting member of the group. Individual variations in the dose required to control symptoms, and differences in sensitivity to side-actions make difficult a final assessment of their comparative values.

The future of the anti-histamines may lie in making better use of the available drugs rather than in the discovery of new drugs. Large numbers of antagonists have been produced in recent years, particularly in the U.S.A., but very few are likely to prove of practical importance. It would, of course, be desirable to find compounds with reduced toxicity and greater duration of action, and an interesting substance for which these properties have recently been claimed is "Histantin" [N-methyl-N'-(4-chlorobenzhydryl) piperazine dihydrochloride] (Jaros *et al.*, 1949 ; Castillo *et al.*, 1949). It is less toxic than pyribenzamine and appears to be almost as long acting as promethazine.

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CHAPTER 4

SENSITIZATION TO DRUGS (DRUG ALLERGY)

The toxic effects which may arise during therapeutic administration of drugs are of two main types :—

(1) Those due to *overdosage*. This is most liable to occur with cumulative drugs like digitalis, or with drugs such as salicylates when given in large doses in the treatment of rheumatic fever.

It should be emphasized that the toxic dose varies considerably among different individuals, a point which is fully discussed in Chap. 17 where the relation between the dose of sodium salicylate and the appearance of signs of salicylism is discussed. Some patients showed signs of toxicity with very small, sub-therapeutic doses. Such individuals might be described as *hypersensitive*, but it must be stressed that the hypersensitivity so revealed relates to the development of symptoms identical with those which would occur in every subject who received a large enough dose.

(2) Those due to *abnormal reactions*, quite distinct from the usual pharmacological or toxicological effects of a drug. Such reactions have been described as "*drug idiosyncrasy*" or "*drug allergy*," and since the characteristic responses may frequently be elicited by very small doses of the drugs, the phrase "*drug hypersensitivity*" has also been used, but clearly in a different sense from that described in the previous paragraph. It is these abnormal patterns of response with which we shall be concerned in this chapter.

Characteristically these reactions are not produced on initial administration of drugs, but only appear after what might be described as an "incubation period"; hence the words "sensitization" or "drug allergy," have been used to describe such responses (the word "allergy" was introduced by Von Pirquet to describe "an altered capacity to react"). In some instances the sensitization reactions which develop in response to the administration of such useful drugs as penicillin, sulphonamides, tridione, and the anti-

thyroid substances, may be so severe as to contra-indicate their therapeutic use. It is an interesting fact that many of the signs of drug sensitization such as fever, leucocytosis, arthralgia, lymphadenopathy and skin rashes, occur also in some infective processes and in serum sickness, in both of which allergic tissue reactions to bacterial and foreign proteins respectively are probably involved. It is important to realize that drugs of quite different chemical constitution, and with quite different pharmacological actions, may produce identical pictures of sensitization.

The subject of drug allergy has been very fully reviewed in relation to skin manifestations by Sulzberger (1940) and by Beerman (1949), and the more general aspects have been discussed by Rackemann (1941), by Longcope (1943) and recently by Sherman (1947 ; 1949). It has been pointed out by these authors that drugs such as adrenaline, caffeine and cascara sagrada have never been shown to cause this type of sensitization, whereas at the other extreme is phenylethylhydantoin (nirvanol) which when given in the treatment of chorea was found to produce signs of sensitization in over 90 per cent of patients. The characteristic features of allergic drug reactions are :—

(1) The reactions which occur are entirely different from the ordinary pharmacological effects, and there is usually an incubation period of 5–14 days after first exposure to the drug.

(2) The symptoms and signs are frequently typical of allergic diseases, e.g. asthma, rhinitis, skin eruptions and fever.

(3) The symptoms and signs can be reproduced on subsequent administration of the drug, sometimes with very small doses.

(4) In certain reactions in the skin, e.g. contact dermatitis, a positive patch test response is obtained after 24–48 hours. In most other cutaneous drug allergic reactions, skin tests are negative.

(5) The reaction is usually specific, i.e. restricted to one drug or to a small number of closely related chemical compounds.

(6) There are usually no demonstrable serum antibodies towards the drug, but the work of Landsteiner and Chase (1942) has suggested that antibodies are present in cells such as lymphocytes.

(7) Rich (1942) has described vascular changes of the periarteritis nodosa type as characteristic of drug and other allergies, but other authors consider that there is no constant histological picture peculiar to allergic reactions.

Other reactions to drugs which are sometimes described as allergic include the production of *hepatitis* by cinchophen, arsphenamines, or sulphonamides, the occurrence of *agranulocytosis* with amidopyrine, sulphonamides, arsphenamines, thiouracil, gold salts and tridione, and the occasional development of *thrombocytopenic purpura* with "Sedormid" (allylisopropylacetylurea). These reactions will not be further discussed here.

Symptoms and Signs of Drug Allergy

Asthma and Rhinitis. In contrast to most other types of drug allergy, asthma and rhinitis usually occur in persons with hereditary predisposition to allergy. Drugs which may produce these reactions include aspirin (acetylsalicylic acid), quinine, sulphonamides, the arsphenamines and penicillin. The asthmatic and urticarial reactions due to aspirin have even been reported as a result of holding a tablet in the mouth for a few seconds, and death has resulted from oral administration of 5–10 grains of this drug (Dysart, 1933).

Urticaria and Angioneurotic Œdema, so commonly seen as manifestations of allergic sensitivity to proteins, may also occur as the result of sensitization to drugs such as salicylates, barbiturates, sulphonamides and penicillin. The *Serum Sickness* type of reaction, most typically produced by injection of foreign sera, can also occur after the administration of drugs. For example, penicillin may produce fever, skin rashes (most frequently urticarial), lymphadenopathy, arthralgia and effusions into joints. These reactions may occur with amorphous or with crystalline penicillin, and appear after an incubation period of 5–14 days, as with serum sickness.

Drug Rashes. Skin eruptions are the commonest, or at least the most frequently recognized manifestations of drug allergy. Almost all types of skin lesion may be produced, and any one drug may produce different types of eruption in different persons. The commonest allergic eruptions are the urticarial and maculopapular types produced by systemic administration of salicylates, quinine, barbiturates, mepacrine, thiouracil, sulphonamides, penicillin and streptomycin. Less usual forms include erythema multiforme and erythema nodosum, the latter of which appears not only in response to drugs like bromides or sulphathiazole, but also as a manifestation of rheumatic or tubercuIous states. A rare but

interesting sensitization reaction is the so-called "fixed drug eruption" which has been fully discussed by Sulzberger (1940). It is seen following systemic administration of certain drugs, e.g. phenolphthalein, phenazone, amidopyrine, barbiturates, the arsphenamines and sulphonamides, and is characterized by the rapid development of one or more sharply circumscribed inflammatory lesions on the skin or mucous membranes, as an invariable response to the administration of the offending drug.

Contact Dermatitis. Local application of substances to the skin may produce sensitization with the development of an eczematous type of eruption, first at the site of application, with perhaps subsequent spread to the skin in other parts of the body. The lesion is primarily located in the epidermis and consists of spongiosis and intraepidermal vesiculation; the vesicles later rupture to produce oozing and dried exudate. In sensitized individuals application of the specific offending substance, as in the patch test, produces the typical eczematous reaction in 24–48 hours.

The development of this type of sensitivity has been much studied in human and in animal skin (guinea pigs). The substance 2 : 4-dinitrochlorobenzene has been used as the sensitizing agent by Wedroff and Dolgoff (1935), Landsteiner and Jacobs (1935) and Sulzberger and Baer (1938). In normal human skin application of a drop of 10 per cent solution of dinitrochlorobenzene produces at most a slight erythema at the end of 48 hours, but after 5–20 days in many persons there appears what is described as a "spontaneous flare-up," i.e. the site of original application shows a typical acute eczematous eruption. This reaction is attributed to the action of residual dinitrochlorobenzene at the original site of application, after an incubation period for the development of the sensitized state. After the "flare-up" it is found that the skin of the whole body has become sensitive and responds specifically to the application of quite low concentrations of dinitrochlorobenzene. Although the skin otherwise appears normal, it may remain in a potentially hypersensitive state for many years.

Many drugs have also been shown to be capable of producing this type of reaction when applied directly to the skin. It is for example sometimes seen when penicillin or sulphonamides, more particularly the latter, are used in the treatment of skin diseases or open wounds. Sulzberger *et al.* (1947) applied sulphonamides

in cream for 12–14 days to the skin of 254 subjects in whom experimental burns had been produced, and observed dermatitis in 49 (19 per cent). The incidence of sensitization was proportional to the water solubility of the compound; sodium sulphadiazine gave the highest figure of 57 per cent, sulphanilamide = 22 per cent, sulphathiazole = 7 per cent and sulphadiazine = 5 per cent. Streptomycin has also been found to produce contact dermatitis in nurses engaged in the preparation and administration of solutions of this substance (e.g. Strauss and Warring, 1947). Interesting recent illustrations of the possibilities of chemical sensitization include reports by Cotter (1946) and Cohen (1949) of the development of skin and other types of allergic sensitization in women after the use of thioglycollic acid in the "Cold Wave" process of hair curling; the occurrence of skin sensitization after repeated applications of Cetrimide (cetyltrimethylammonium bromide) (Cruickshank and Squire, 1949) and the development of allergic dermatitis after topical use of antiseptic organic mercurial compounds, e.g. thiomersalate (Gaul and Underwood, 1949).

It should be emphasized that in some persons systemic administration of drugs may lead to sensitization of the contact dermatitis type, and on the other hand topical application may produce sensitization which can be elicited by oral administration of the drug, and may include constitutional reactions such as fever.

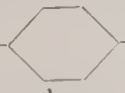
Drug Fever. Febrile reactions have been clearly recognized as an allergic response to many drugs such as iodides, nirvanol, thiouracil, mepacrine, sulphonamides, penicillin and streptomycin. The temperature may rise to high levels, e.g. 105 F., and the fever is frequently accompanied by maculopapular skin rashes. The reaction usually develops after 7–10 days of drug administration, but if a particular drug has been given previously sensitization may already be present, and drug fever may then follow the first doses of the drug, as has been frequently reported with the sulphonamides. It is important to appreciate the possibility that a sharp rise in temperature may be due to drugs, as continued administration of the causal drug occasionally leads to disastrous results. Deaths have been reported following such reactions with sulphonamides, especially sulphathiazole, and necropsy has revealed widespread lesions resembling periarteritis nodosa (Rich, 1942) as well as focal necroses of myocardium, liver, bone marrow,

spleen, lymph nodes, kidneys and other organs (Simon, 1943; More *et al.*, 1946; French, 1946). Similar lesions have resulted from sensitization to iodine, thiourea and thiouracil.

Specificity of Sensitization Reactions

It is obviously important to know whether, or to what degree, sensitization reactions to drugs are restricted to the drug originally concerned with the allergic response. Among the sulphonamides, some patients sensitized to one drug have not reacted to other members of this group (Dowling *et al.* 1941), other patients have reacted to sulphadiazine, sulphathiazole and sulphapyridine, but not to the parent substance, sulphanilamide (Sherman and Cooke, 1947). Others have shown sensitivity to all members of the sulphonamide group, and indeed in some instances the sensitization has extended to other p-aminobenzene compounds such as sulphanilic acid, p-aminobenzoic acid and procaine (Sulzberger *et al.* 1947; Park, 1944). In spite of this evidence of cross-sensitization, it is generally agreed that the allergic responses are greatest in response to the original sensitizing drug; for example Dowling *et al.* (1946) observed reactions in 69 per cent of sensitized subjects when the original drug was readministered, and in only 17 per cent when some other sulphonamide was given. These latter findings are in agreement with the experimental work of Wedum (1942) who sensitized guinea pigs and rabbits to sulphonamide azoproteins. In guinea pigs, tests for skin sensitization and for lethal anaphylactic shock, and in rabbits, tests for serum precipitins, all showed the close immunological relation between the different sulphonamides, but in each test the sulphonamide azoprotein to which sensitization had been originally produced gave the most marked response.

Variations in specificity of response have been described in relation to other drugs producing sensitization. For instance, Goodman (1939) found in a patient with procaine dermatitis, that sensitization existed towards compounds containing the



COO-group, but not towards cocaine, or quinoline compounds such as cinchocaine. Other examples of "group" sensitization include the observations of Dawson and Garbade (1930) who, in a quinine sensitive person, noted reactions to seven related lævo-rotatory compounds, but none towards the corres-

ponding dextro-isomers ; sensitivity towards arsphenamine usually extends only towards other organic trivalent arsenical compounds, but sometimes also towards organic pentavalent arsenicals (Epstein, 1937).

In other cases specificity of response is remarkably restricted. Cooke (1919) observed in aspirin sensitive patients no responses to salicylic acid, benzoic acid or methylsalicylate, and Horsfall (1934) found that a patient extremely sensitive to formaldehyde, gave no reactions to other aldehydes or to formic acid or methyl alcohol. Rostenberg and Kanof (1945) studied the skin responses in persons experimentally sensitized to 2 : 4-dinitrochlorobenzene, towards a number of isomers and closely related compounds. The corresponding bromo- and iodo-compounds reacted like the chloro- compound, and the 2 : 5- and 3 : 4-dinitro-compounds were the most active isomers. There was very little response to 2 : 4 dinitro-amino-benzene.

Factors Influencing Development of Drug Sensitization

Individual Variation. It is clear that there must be considerable individual differences in susceptibility to account for the occurrence of allergic responses in only about 10 per cent of patients who receive drugs such as the sulphonamides, and thiouracil and its derivatives, in full therapeutic doses. The mode of administration, the dosage of drug, and the presence of certain local lesions may be contributing factors in some cases, but the experimental human studies of Landsteiner *et al.* (1939) have revealed inherent differences in susceptibility of the skin towards sensitization by p-nitrosodimethylaniline (A) and by 2 : 4 dinitrochlorobenzene (B). The results were as follows :—

50 of 82 persons were sensitized to A or B or both.
21 persons were equally sensitized to A and B.
20 persons were sensitized to B more than to A.
9 persons were sensitized to A more than to B.

In some subjects high susceptibility towards one compound was associated with little or no response to the other. These results are particularly interesting, since it is most improbable that any of the persons studied had been previously exposed to these particular, or other closely related, compounds, so the sensitizing capacity was being tested on a virgin field.

Drug Dosage. It has sometimes been assumed that there is no relation between drug dosage and liability to sensitization, but this view has recently been challenged by Lehr (1948) who has studied the subject in relation to the sulphonamides. From figures obtained from the literature he has shown that increasing dosage leads to greater frequency of sensitization reactions; for example, in the case of sulphathiazole 2–4 G. daily for 3–15 days produced no cases of drug rash or fever in 3,584 patients; 6 G. daily for 3–10 days produced over 6.4 per cent of reactions in 2,475 cases, and 6–10 G. daily produced more than 10 per cent of such reactions. Similar differences were noted with increasing doses of sulphanilamide and sulphadiazine. Lehr suggests that sensitization is most unlikely to occur with dosage of less than 2 G. of sulphonamide daily (with blood levels of less than 5 mg./100 ml.). He has also reported some preliminary clinical experiences in which he has administered mixtures of 2 or 3 sulphonamides (e.g. sulphadiazine, sulphathiazole and sulphamerazine) so that not more than 2 G. daily of any individual drug were given. Lehr's results suggest that such mixtures not only reduce the liability to crystal formation in the urinary tract, but also diminish the tendency to develop drug sensitization. He quotes evidence which suggests that the principal toxic effects of the thiouracil compounds are also related to dosage, since the smaller doses of methyl- and propyl thiouracil (50-200 mg. daily) now used, lead to fewer allergic side effects than were noted formerly with doses of thiouracil up to 1 G. daily.

Duration of Drug Sensitization

In some cases it has been clearly established that sensitization to drugs can persist for many years, but in other cases the hypersensitivity may fluctuate or perhaps last only a few months. Indeed in the case of penicillin, Hopkins and Lawrence (1947) found that of 27 subjects who gave positive intradermal reactions to penicillin, 8 had become negative within 2–12 weeks, and others have confirmed the transient nature of penicillin sensitization (to the amorphous or crystalline forms) in the therapeutic use of the drug.

Desensitization

By analogy with other allergic states, in which specific desensitization procedures are sometimes strikingly successful, as

in hay fever, attempts have been made to desensitize patients who have developed any of the above described signs of drug allergy. Park (1944) and Tate and Klorfajn (1944) have reported successful results in some patients sensitive to sulphonamides, to whom they gave initial doses of 0.1–0.125 G. of the responsible drug, and subsequently increased the dosage until normal amounts could be tolerated. In some patients with sulphonamide dermatitis it may take several weeks to desensitize, so these procedures have not been widely employed.

Mechanism of Drug Sensitization

The mode of development of the sensitized state to drugs, and the manner in which the reactions are elicited, are probably related to the general mechanisms responsible for the similar phenomena recorded with foreign and bacterial proteins. It has been generally assumed that only proteins can act as antigens and that the specificity of the induced sensitization is due, in most instances, to the structure of the molecule as a whole. Landsteiner, whose extensive work on antigen-antibody reactions has been summarized in his book "The Specificity of Serological Reactions" (1946), has shown, however, that whilst a protein molecule may be necessary for the formation of a complete antigen, the specificity of the induced states of immunity or sensitization may be due to some small molecular component, called a *hapten*, which can be regarded as the prosthetic group of an active protein (see Chap. 15). The hapten is by itself incapable of either initiating antibody formation or eliciting antigen-antibody interactions, but when suitably conjugated with proteins, the complex antigen so formed can do both. A hapten may, however, inhibit serological reactions such as precipitation, by combining specifically with antibody receptors and thus preventing access of the complete antigen.

Landsteiner and his colleagues used artificial conjugated antigens to reveal many of the features of specific interaction between antigens and antibodies, and some points are of great interest in relation to drug allergy.

If certain azo-dyes, themselves non-antigenic, were first conjugated with proteins, the conjugated substances could produce anaphylactic sensitization in guinea pigs, and in some instances it was shown that anaphylactic shock could be elicited specifically not only by the conjugate, but also by the azo-dye alone (Landsteiner

and Van der Scheer, 1933). It was next suggested that hapten-protein complexes analagous to these azo-proteins might be formed in the body (particularly in the skin) by interaction between sensitizing agents, acting as haptens, and tissue proteins. In some cases of experimental sensitization, combination of simple chemical substances with body proteins is highly probable. For example, p-phenylenediamine, well known as a sensitizing agent in the production of fur dermatitis, is readily oxidized, and the oxidation products combine with proteins to form deeply coloured compounds comparable to azo-proteins (Mayer, 1938). Further support for such *in vivo* formation of antigenic protein conjugates was provided by the studies of Landsteiner and Jacobs (1935) and Sulzberger and Baer (1938) on skin sensitization in man and animals to chloro- and nitro-substituted benzenes; it was found that with compounds of this type, sensitizing capacity was closely related to capacity to interact with the amino group in aniline, and it was suggested that similar reactions with free amino groups in protein might form antigenic conjugates; other compounds found capable of producing sensitization in guinea pigs included acid chlorides and acid anhydrides, all of which combine readily with proteins. It is known that many drugs which can cause sensitization, e.g. arsphenamines, sulphonamides, and penicillin, combine with plasma proteins and if it is postulated that attachment to tissue proteins, particularly in the skin, can also occur, the complete antigen thus formed is clearly comparable to natural protein antigens. The work of Landsteiner *et al.* and that of Gell (1944) have shown that sensitization produced by intracutaneous injection in guinea pigs of compounds such as picryl chloride or "tetryl" (N-methyl, N-nitroamino-2 : 4 : 6-trinitrobenzene) leads not only to skin hypersensitivity, shown by application of the simple compound directly to the skin, but also to anaphylactic hypersensitivity, revealed by application of picryl proteins to the isolated gut or uterus. The anaphylactic sensitivity is associated with the development of specific antibodies, passive transfer by serum is obtainable, and desensitization can be produced. Contact dermatitis has never been transferred by serum, and it is impossible to desensitize towards this reaction by local application of the allergenic compound, though Chase (1946) has been able to inhibit the development of experimental drug allergy in guinea pigs by preliminary feeding of skin sensitizing substances.

Landsteiner and Chase (1942) were able to transfer skin sensitivity of the contact dermatitis type in a most interesting and ingenious manner. In guinea pigs sensitized to picryl chloride, a peritoneal exudate was produced by intraperitoneal injection of dead tubercle bacilli or tuberculin; subsequent intraperitoneal injection of the cellular sediment of this exudate into normal guinea pigs produced the characteristic skin sensitivity to picryl chloride in about two days, and this sensitized condition persisted for a few days.

To sum up the experimental work on the development of contact dermatitis, the following speculative scheme might be suggested:—

First, the sensitizing agent must penetrate the epidermis before it can combine with the appropriate protein to form the complete antigen. The work of Landsteiner and Chase (1939) suggests that the next step is the transport of the complete antigen via lymphatics to the regional lymph nodes, where there is a delay of some days while antibodies are formed within the newly manufactured lymphocytes. These cells would then be distributed via the blood stream to the various body tissues. Subsequent application of the sensitizing substance would form fresh antigen which would then react with the antibody-containing lymphocytes, or with antibodies deposited from the lymphocytes in the skin itself, to produce the characteristic inflammatory response in 24–48 hours. Other types of drug sensitization might involve antigen formation or antibody deposition, or both, in tissues other than the skin.

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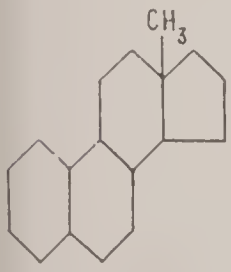
CHAPTER 5

THE STEROIDS OF THE GONADS AND ADRENAL CORTEX

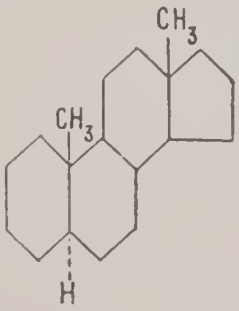
Chemistry

The general structure of steroids was finally elucidated some fifteen to twenty years ago (Fieser and Fieser, 1949) and this made possible the rapid advances in our knowledge of the steroid hormones and related substances. Since the chemistry of these substances is rather confusing it is useful to start with the main compounds on which the structure of the various hormones is based. Selye (1942) discusses their properties in detail.

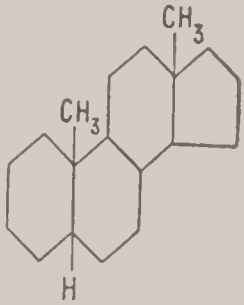
All these compounds can be derived from three saturated parent hydrocarbons, namely œstrane, androstane and ætiocholane. Certain C_{17} alkyl substituted derivatives of these substances are often referred to in the literature by special names, e.g. pregnane for 17-ethyl-ætiocholane, and *allo*-pregnane for 17-ethyl-androstane. Common names are also used for several substances which are hormones or their derivatives, e.g. œstradiol, progesterone and testosterone. The structure of the compounds and that of cholesterol, as well as the letter used for the description of the various rings and the numbers used to designate the various carbon atoms are given below :—



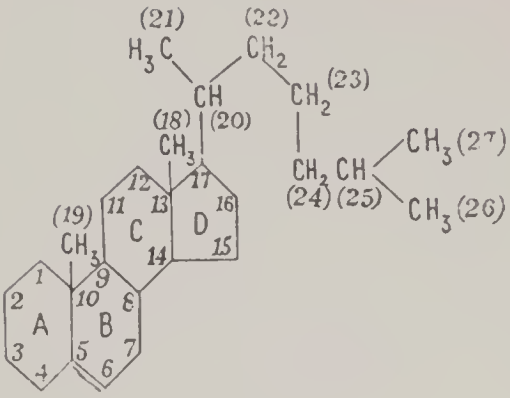
Œstrane



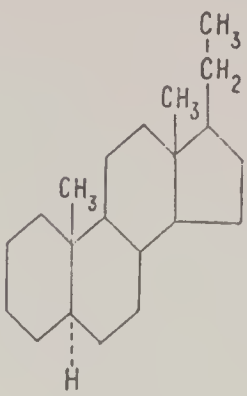
Androstane



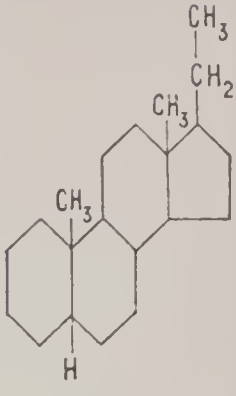
Ætiocholane



Cholesterol

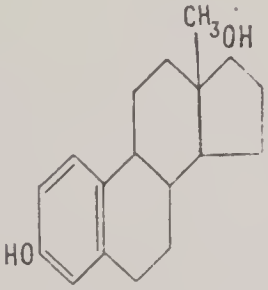


17-ethyl-androstane (Allo-pregnane)

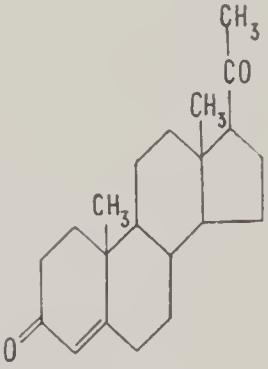


17-ethyl-ætiocholane (Pregnane)

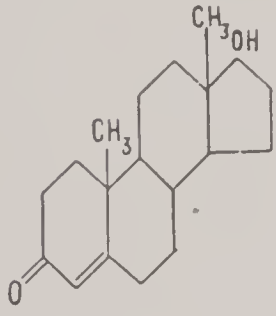
The main steroid hormones of importance in the body are œstradiol, progesterone, testosterone and deoxycorticosterone (of which the B.P. name is deoxycortone), and their chemical constitution is given below :—



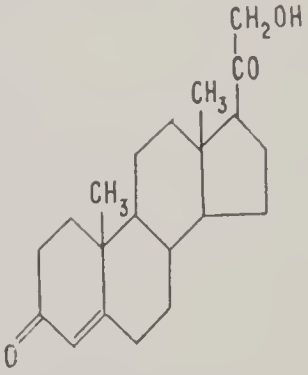
Œstradiol



Progesterone



Testosterone



Deoxycortone

These hormones can be partially synthesized from certain other steroids, e.g. from cholesterol. Œstradiol can be produced from œstrone and the complete synthesis of the latter substance has been described by Anner and Miescher (1948) and has also been reported by Breitner (see Kleiderer *et al.*, 1945).

Œstradiol and progesterone are secreted by the ovary. Testosterone is produced by the interstitial cells of the testes, and deoxycortone is found in the cortex of the adrenal glands, and is probably one of the many steroids elaborated there. In fact 28 steroids, including œstrone and progesterone, have now been isolated from the adrenal cortex, but it is not known how many of these are naturally secreted there and how many are artefacts produced during the process of extraction (Reichstein and Shoppee, 1943; Shoppee, 1947). Only eight of these substances possess physiological activity, including deoxycortone, corticosterone, and 11-dehydrocorticosterone. The greater part of the activity of adrenal cortical extracts is contained in an amorphous residue of which the active principles, probably also steroids, have not yet been isolated.

All these substances obtained from the gonads and adrenal gland are responsible for controlling the changes in the secondary sex organs and for maintaining the endocrine functions of the adrenal cortex. The effects produced by the three sex hormones are described in detail elsewhere (e.g. Robson, 1949; Chap. IV–VI). Obviously the production of these hormones in the body constitutes merely one aspect of their function, which cannot be properly exerted unless the tissues affected are receptive to their action.

These steroid substances and some of their derivatives may overlap in their actions in the body. Thus for example many of the effects which are produced by progesterone are also brought about by deoxycortone. It seems likely that this overlapping in the action of some of these substances is due to two factors. (1) Some substances produce a certain effect in the body but in the course of their metabolism another substance is produced which has a different effect. For example, ethisterone has progesterone-like actions and, in fact, when applied directly to the uterine endometrium, it is nearly as active as progesterone. It can also produce œstrogenic effects but this only occurs after a metabolic change in the body, ethisterone itself not being œstrogenic (Emmens, 1941). (2) Some steroids have two quite distinct actions. As an example, deoxy-

cortone maintains life in adrenalectomized animals, but it also has a progesterone-like action on the uterus since it produces its specific effect on the endometrium locally when implanted into the lumen of this organ.

Origin of Steroid Hormones

There is good reason to believe that the steroid hormones are derived in the body from cholesterol. Strong support for this view is given by the work of Bloch (1945) with cholesterol containing deuterium (heavy hydrogen). He administered this substance to a pregnant woman and found that the pregnanediol recovered from the urine contained a concentration of deuterium similar to that present in the cholesterol circulating in the blood. Since progesterone is, to some extent, converted to pregnanediol, which is then excreted in the urine, this means that the luteal hormone was probably derived from cholesterol. This view is also supported by the findings of Claesson and Hillarp (1947) who by means of polarization, optical and histochemical methods demonstrated the presence of a steroid substance, probably cholesterol, in the ovary. The store of this substance is depleted when œstrogen is secreted. These authors regard the interstitial cells of the ovary of fundamental importance in the formation of œstrogen. By cholesterol estimations in the gonads, evidence has also been obtained that this substance may be a precursor of progesterone in the female (Perlman and Leonard, 1947) and of androgen in the male (Tepperman and Tepperman, 1947).

It is still uncertain how cholesterol is converted into these various substances, but the first step probably involves the breakdown of the long side chain attached to C_{17}. The question is discussed by Fieser (1937), Marker (1938) and Pincus and Pearlman (1943). Marrian (1939) has suggested that the steroids of the adrenal gland may be formed from progesterone, which he believes the gland produces in large quantities. It is possible, too, that the steroid hormones can also be built up from smaller molecules, since it has been found that deutero cholesterol is formed when sodium deutero acetate is fed to mice and rats (Bloch and Rittenberg, 1942). Even more interesting is the finding of Srere *et al.* (1949) that beef adrenal cortex can synthesize cholesterol from acetate *in vitro* : the acetate was labelled with carbon 14 which was then detected in a constant proportion in the cholesterol formed.

Fate of Steroid Hormones

These substances are rapidly metabolized in the body and most of their activity is thereby destroyed. There is good evidence that the liver is chiefly involved in the destruction of œstradiol, and that it also plays a part in the destruction of progesterone and testosterone. A number of steroid metabolic products are formed which, with the exception of pregnanediol, account for only small fractions of the parent compounds. These substances are excreted in the urine either as such or in a conjugated form. Some of the derivatives are conjugated with glucuronic acid, but conjugation with sulphuric acid also occurs, e.g. in the case of œstrone (Schaehter and Marrian 1938) and of dehydroisoandrosterone (Munson *et al.*, 1944).

The changes which may occur in the degradation of the steroids are discussed by Doisy (1942), Koch (1942) and Pineus and Pearlman (1943). It is likely that specific enzymes are concerned with those processes but they have not yet been identified. There is evidence that cozymase may be involved (Coppedge *et al.*, 1948). The isolation of metabolic products of the steroid hormones in the urine is of interest not only because it may shed light on the fate of the hormones in the body, but also because it may serve as an index of the amount of hormones produced in the body. The main substances excreted are œstrone and œstriol, derived from œstradiol; pregnanediol derived from progesterone; and androsterone, dehydroisoandrosterone, and other substances, some with androgenic properties, derived from testosterone and from the adrenal cortex (Schiller *et al.*, 1945). Various methods have been elaborated for the assay of these substances in the urine and the results so obtained have been used to estimate the extent of ovarian, placental, testicular, and adrenal cortical activity. There are, however, a number of difficulties which are only slowly being overcome. For example the amount of metabolite in the urine is only a small, and not necessarily a constant, fraction of the parent substance. An examination of the many data on the excretion of various substances following the administration of œstradiol, progesterone, etc., in animals and man shows this quite clearly. The results with progesterone are particularly instructive. For a time it was believed that this substance was almost quantitatively converted into pregnanediol but more recent work shows that probably only about 25 per cent of the hormone appears in the urine in this form. In the case of adrenal cortical hormones the

amounts produced by the gland have been estimated (Vogt, 1947) and have been found to be very large in comparison with the quantities excreted in the urine (or extractable from the gland).

Changes in the rate of metabolic degradation of the steroids may occur. It has, for example, been suggested that nutritional conditions, and especially the supply of some parts of the vitamin B complex, may influence the metabolism of œstrogens by the liver, and that a deficiency of vitamin B may indeed lead to deficient œstrogen inactivation and thus to the development of a number of clinical abnormalities in both sexes (see discussion by Biskind and Biskind, 1946).

A further difficulty is that the origin of the various substances found in the urine is by no means always clear. Much of the œstrogen is probably derived from the ovary and, in pregnancy, from the placenta. But some œstrogen is excreted in ovariectomized women in whom it is probably produced by the adrenal cortex, which is also responsible for some of the œstrogen found in the urine of the male. In some species, e.g. the stallion, very large quantities of œstrogen are secreted by the testes and excreted in the urine. It is quite likely that in the human male, too, the testes secrete some œstrogen.

The same difficulty applies to the excretion of androgenic substances in the urine. In the male much of the urinary androgen consists of metabolic products of testosterone but some is undoubtedly derived from the adrenal cortex, and this fraction is still found in the urine after castration. As will be seen below, attempts have been made to differentiate between the adrenal and testicular androgen in the urine. Adrenal cortical metabolic products appear as androgen in the urine of women but it is possible that the ovaries also contribute their quota, since there is evidence that the female gonads can secrete some androgen.

Most of the pregnanediol in the urine is no doubt derived from progesterone produced in the ovaries and placenta, but it is quite likely that some of it represents a metabolic product of steroids elaborated in the adrenal cortex. Pregnanediol can be excreted in men (Westphal, 1944) and in bulls (Marker *et al.*, 1938). It may be derived from adrenal progesterone and deoxycortone. Both these substances are present in the gland and their administration in men is followed by an excretion of pregnanediol (Buxton and Westphal, 1939; Cuyler *et al.*, 1940).

Assay of Steroid Hormones

The effective fractionation of urine for its various steroid constituents is not easy and no standard procedures have yet been established. Methods are reviewed by Pincus (1945). The various fractions must be assayed by chemical or biological methods, or both. When large quantities of these substances are excreted the results are reasonably accurate but no really satisfactory methods have yet been elaborated for the assay of small amounts of any of the products excreted in the urine, and this has seriously limited the value of such methods in connection with the diagnosis and treatment of endocrine diseases.

Recent work has been mainly concerned with the elaboration of new methods for (1) the chemical estimation of the steroids and (2) the biological assay of some of these substances in small amounts. For a detailed review the reader is referred to the book edited by Emmens (1950) on "Assays in Hormone Research."

(1) *Chemical Methods :* A number of modifications of the Kober method for the assay of *natural œstrogens* have been introduced involving essentially more accurate measurements of the colour formed and an elimination of interfering factors. Other methods have also been introduced. The section by Haslewood in the book mentioned above should be consulted.

The chemical assay of *androgens* in the urine is based on the Zimmermann colorimetric reaction. This is applied to the fraction of the urine which contains steroids with a ketone group in position 17 (hence these substances are called the 17-ketosteroids). Androsterone and dehydroandrosterone are chiefly involved (Pincus, 1943) and it has been shown that there is a significant degree of correlation between the values obtained by the colorimetric assay of such fractions and those values obtained by the biological assay on the capon and expressed in International Units of male hormone (Callow *et al.*, 1938). Improvements on the Zimmermann technique have been suggested by Talbot *et al.* (1942) and by Cahen and Salter (1944). A new colorimetric method based on the development of a blue colour with antimony trichloride in the presence of acetic acid has been described by Pincus (1943) and a polarographic method, which gives rather smaller values than those obtained with the Zimmermann reaction, has been elaborated by Barnett *et al.* (1946). Full details are given in the section by Callow in "Assays in Hormone Research."

Attempts have also been made to estimate substances specifically derived from the *steroids produced in the adrenal cortex*. A test for these substances is based on the reduction of phosphomolybdic acid (Heard and Sobel, 1946; Heard *et al.*, 1946) and the results obtained agree with those obtained by a biological method of estimation, in which the extract is administered to glucose treated adrenalectomized mice and the deposition of glycogen in the liver measured (Venning *et al.*, 1946). The excretion of these substances does not vary with that of the 17-ketosteroids (Venning and Kazmin, 1946), supporting the view that they are not derived from the gonads but from the adrenal gland.

Much work has been done on the estimation of *pregnanediol* in the urine, as a satisfactory method would be of value in determining whether ovulation and corpus luteum formation had occurred during the second part of the menstrual cycle, and might also serve as an easy method of pregnancy diagnosis. Henderson *et al.* (1949) describe a modification of the Astwood-Talbot method which, they claim, will detect amounts down to 0.3 mg. in 100 ml. of urine and takes $3\frac{1}{2}$ hours to carry out. They describe the application of this method to the estimation of luteal function during the menstrual cycle and pregnancy. The subject is reviewed in the section by Haslewood in "Assays in Hormone Research," and also by Swyer (1949) who concludes that, in general, pregnanediol estimations are of little value to the practising clinician.

(2) *Biological Methods :* The assay of small amounts of œstrogen, androgen and progestogen depends on the direct application of the material to be assayed to a suitable test object. Such local effects can easily be obtained, as a rule, but it is much more difficult to make such a reaction quantitative.

In the case of *œstrogens* the material is introduced into the vagina of ovariectomized mice and positive responses can be obtained with very small doses, e.g. 0.001 μg. or even less (Mühlbock, 1940). Positive changes in the vagina not involving full cornification are, however, produced by doses covering a rather wide range, i.e. from less than 0.001 μg. to more than 0.01 μg., and a good deal more work is necessary to make such a test really quantitative.* Small amounts of *androgen* can be assayed by

* C. W. Emmens (1950, *J. Endocrinol.*, 6, 306) has shown that it is possible to assay œstradiol and œstriol in terms of the international standard by the intravaginal method.

applying the material directly to the surface of the comb of the capon. The test is about 100 times as sensitive as when the material is injected into the animal, and definitely positive effects are obtained with 1 μg. of androsterone. This method has been successfully applied to the determination of the androgen excretion in normal men and women (Hamburger *et al.*, 1945). Small amounts of substances with a *progesterone*-like effect can be assayed by local application to the uterus. McGinty *et al.* (1939) and Haskins (1939) used immature rabbits previously treated with œstrogen and introduced a solution of the substance to be tested into the lumen. Positive responses may be obtained with doses as small as 0.25 μg., but they may also occur in parts of the uterus not treated with the progestogen. When this material is introduced as a solid into the uterine lumen of mature ovariectomized animals pretreated with œstrone, the response is restricted to the implanted area (Robson and Höhn, 1947), and effects can be obtained with doses of progesterone of the order of 1 μg. Other progestogens, e.g. ethisterone, pregnenolone, also produce a local effect (Robson and Höhn, 1950). A method of assaying progesterone which involves a local action on the uterus of the mouse is described by Hooker and Forbes (1947).

Functions of the Adrenal Cortex

Much work has been done in order to explain the mechanisms by which the adrenal cortex produces its effects in the body, but at present there is no simple theory which will explain the large number of experimental findings. Verzár (1939) believes that the adrenal hormones play a vital part in the cellular metabolic processes and are particularly concerned in phosphorylation mechanisms (Schumann, 1940; Verzár and Montigel, 1942), and the investigations of Cori and her co-workers (Price *et al.*, 1946) and of Conway and Hingerty (1946) support this view.

The adrenal cortical hormones have two main effects, viz. on *electrolyte* and *water metabolism*, and on *carbohydrate metabolism*. Kendall (1948) reviews the influence of the adrenal cortex on the metabolism of water and electrolytes. It is one of the primary actions of the cortical steroids to influence the renal excretion of Na, Cl, and K ions, probably by affecting their tubular reabsorption. In untreated adrenalectomized animals there is increased excretion of Na and Cl and retention of K; there is loss of Na from

muscle and a rise in the K content of tissue cells and plasma. After a time the ability to excrete administered water is lessened. It is to be noted, however, that good health and normal growth can be maintained in adrenalectomized animals if sufficient NaCl is administered. Administration of deoxycortone acetate to adrenalectomized animals stops the loss of sodium chloride, and such animals can be maintained in good health even on diets with a low content of sodium chloride; this steroid also causes an increase in the excretion of potassium. These effects on the electrolyte balance are also seen when deoxycortone acetate is administered to patients suffering from Addison's disease.

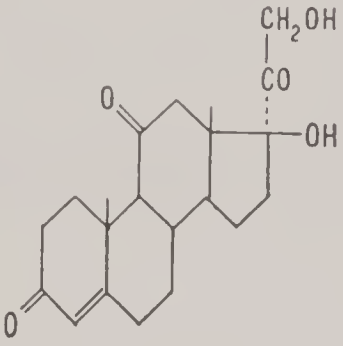
There is also an important effect on carbohydrate metabolism. In adrenal insufficiency, animals are unable to store administered carbohydrate in the form of glycogen as rapidly as normal animals, particularly in the liver, and an unduly high proportion of the carbohydrate taken in is oxidized. It has been suggested that protein metabolism is also directly affected, but there is at present no definite evidence that such is the case (Ingle and Oberle, 1946; Leathem, 1945). An attempt has been made to correlate these actions with the chemical constitution of the adrenal steroids (Shoppee, 1947). Effects on the carbohydrate metabolism appear to be associated with an oxygen atom (hydroxyl group or ketonic oxygen) at position C_{11} (e.g. 17-hydroxy-11-dehydrocorticosterone), while the presence of a tertiary hydroxyl group at C 17 diminishes the capacity to maintain life, and to retain NaCl in the body.

There is much evidence that the adrenal cortex is associated with the protection of the body against harmful stimuli, and also with the control of lymphoid tissue and especially with the release of antibody from lymphocytes (Dougherty *et al.*, 1945). The question is discussed by Ungar (1947) who states quite rightly that much hard work and clear thinking will be required before a satisfactory interpretation can be given for the experimental findings. Stresses of various types produce a response in the body to which the term *adaptation syndrome* has been given by Selye (1946). The development of the adaptation syndrome can be divided into three stages, namely (1) the *alarm reaction* which is the sum of all non-specific systemic phenomena elicited by sudden exposure to stimuli to which the organism is not adapted, (2) *adaptation* proper, which can be defined as a rise in the threshold of action of stimuli following the application of the same or another

stimulus, and (3) the *exhaustion* phase which follows very prolonged exposure to stimuli to which adaptation has been developed, but can no longer be sustained. Selye claims that the adrenal cortex plays an important part in this process of adaptation, and that the pituitary and spleen may also be concerned. Abnormalities in the processes of adaptation can apparently lead to pathological conditions to which Selye (1949) has applied the term "diseases of adaptation."

Cortisone in Rheumatic Diseases

A most interesting and important development in the clinical application of cortical steroids is the use of *cortisone* (17-hydroxy-11-dehydrocorticosterone; Kendall's "compound E"; Reichstein's "Fa 6"; Wintersteiner's "F") in the treatment of rheumatic diseases. Only preliminary results are available at present, but they are very striking.



Cortisone
(17-hydroxy-11-dehydrocorticosterone)

This work was initiated at the Mayo Clinic by Hench who discusses the reasons which led him to this investigation in a separate paper (Hench, 1949). He was struck by the fact that rheumatoid arthritis is not infrequently relieved by pregnancy, or by the development of hepatitis with jaundice. Many measures were tried to reproduce similar biochemical conditions in patients suffering from rheumatoid arthritis, e.g. transfusion of blood from jaundiced and pregnant donors, the artificial production of jaundice by the injection of icterogenic serum, or by the oral administration of lactophenin. Hench came to the conclusion that the antirheumatic agent might be an adrenal hormone, since temporary remissions of rheumatoid arthritis are frequently

induced by procedures which are known to be capable of stimulating the adrenal cortex, e.g. general anæsthesia and surgical operations.

In September 1948 cortisone became available for clinical trials. Its partial synthesis from desoxycholic acid on a scale suitable for clinical work had been reported by Sarett (1946). Previous partial syntheses had been described in 1936 by Kendall and his co-workers and in 1937 by Reichstein. The clinical results are described by Hench *et al.* (1949).

Mode of Administration

The material has been given as small crystals, 5–10 micron in size, suspended in saline (25 mg. per ml.). In the earlier cases the method of production of the compound had not been fully established and constant results were not obtained. Later material, prepared according to satisfactory physical and chemical criteria, has given more satisfactory results, with no notable toxic effects. Later still it was found that the acetate of the steroid is easier and less expensive to prepare, and this is now being used. The dose of cortisone is 100 mg. per day by intramuscular injection. With the acetate, which is absorbed rather more slowly, the initial dose is 300 mg. and this is followed by 100 mg. doses per day.

Clinical Results

The compound was administered to 14 patients with severe or moderately severe rheumatoid arthritis. In each of the 14 patients the initial results were as follows as described by the authors: "Within a few days there was a marked reduction of stiffness of muscles and joints, lessening of articular aching or pain on motion and tenderness, and significant improvement of articular and muscular function." The appetite often rapidly improved and several patients gained weight. A feeling of well being was also noted.

The treatment also consistently produced a fall in the sedimentation rate. Sometimes this occurred promptly and rapidly, and sometimes more slowly (see Fig. 9). Generally normal rates appeared within 10 to 35 days.

In nine patients the treatment was given for only 8 to 61 days. Then, unknown to the patients, injection of the hormone was replaced by injection of a control preparation (100 mg. of choles-

terol per day). In eight out of nine cases symptoms began to return or to increase very quickly, generally within 2–4 days. The sedimentation rate usually also increased rapidly. Similar striking results in a small number of cases have been reported by a number of other investigators. Prolonged administration of these large doses of cortisone may lead to the development of a

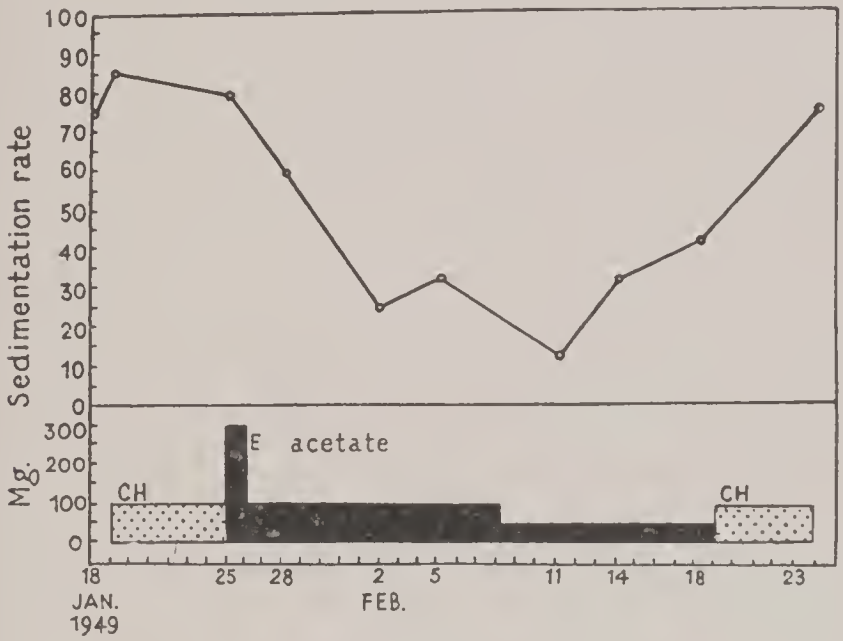


FIG. 9

Effect of cortisone on the sedimentation rate of a 44 year-old woman with severe rheumatoid arthritis. Note that at first the sedimentation rate was not affected by the control solution CH (cholesterol); then the rate decreased markedly when an adequate dose of cortisone was used; then it increased when the dose of cortisone was reduced; finally, when the use of cortisone was discontinued and cholesterol was substituted, the sedimentation rate increased markedly.

(After Heneh *et al.*, 1949.)

Cushing-like syndrome, and allied compounds are now being investigated in the hope of finding one which will not have this effect. Of interest too is the finding that wounds show no signs of healing, and abseesses no signs of granulation tissue formation, in patients (or animals) to whom cortisone (or ACTH) is being administered (see McNee, 1950).

As the work on cortisone develops it is becoming evident that this substance is of value not only in the treatment of rheumatoid arthritis but also in a number of other conditions, involving the collagen tissues of the body. Thus it is believed to be of benefit in rheumatic fever, metastatic carcinomatosis, hyperpiesia, the shock of coronary thrombosis and also in gout (see *Brit. Med. J.*, 1949, ii, 166). The effects in rheumatic fever are described by Hench *et al.* (1949). They administered cortisone to three patients during the acute stages of the disease; this was followed by a rapid disappearance not only of the fever, tachycardia and polyarthritis, but also of the elevated sedimentation rates and abnormal electrocardiographic changes. The authors also suggest that cortisone may produce a beneficial effect on the cardiac muscle and valves in rheumatic fever, in view of its action on skeletal muscle and fibrous tissues in patients with rheumatoid arthritis.

Among the incidental observations in this investigation are the findings that the administration of cortisone increases the urinary excretion of corticosteroids, and decreases the excretion of 17-ketosteroids.

Cortisone has also given striking results in the treatment of Addison's disease (Forsham *et al.*, 1949). Fourteen patients were treated and marked clinical improvement followed the administration of 50–100 mg. of the acetate daily for 2–6 days. The implantation method gave even more satisfactory results and showed that the actual daily need of the substance is much lower. Five patients were implanted with five to ten 50 mg. pellets and the clinical improvement was sustained for several months. Absorption averaged only 0.5 mg. per pellet per day, i.e. the daily dose was 2.5–5 mg. A number of metabolic studies were made and it was found that the administration of cortisone produced a small but definite retention of sodium chloride, an increased uptake of radioactive iodine by the thyroid and a transient rise in fasting blood ketone bodies. The last results were also obtained by Bennett *et al.* (1949), who conclude from their studies that cortisone facilitates the breakdown of fat via ketone bodies. Awapara *et al.* (1949) found that cortisone promotes the deposition of glycogen in the liver of adrenalectomized rats, and also studied the effect on the amino acid content of liver and muscle. Obviously extensive studies on the metabolic actions of cortisone in relation to its clinical effects are required.

Results with Adrenocorticotrophic Hormone (ACTH)

This anterior pituitary hormone stimulates the adrenal cortex and would thus be expeeted to inerease the rate of seeretion of cortisone and of some other cortieal steroids. There is indeed good evidence that the administration of eortieotrophin in man is followed by an increased production and excretion of steroids derived from the adrenal cortex (Mason *et al.*, 1948 ; Forsham *et al.* 1948). In rats corticotrophin affects the seeretion of those adrenal steroids which influence sugar metabolism, but not of those which affect electrolyte balance (Bergner and Deane, 1948). The properties and actions of ACTH have been reviewed by Li (see *Lancet*, 1950).

Corticotrophin was tried by Hench and his co-workers in two patients with rheumatoid arthritis who reeeived 100 mg. of the hormone preparation daily for 12 days, by intramuscular injection. The results were as striking as with cortisone. There was marked clinical improvement and a prompt fall in the sedimentation rate, which rose again after cessation of treatment. There were definite side effects, including a sense of exhaustion and a moderate rise in blood pressure, possibly due to small amounts of posterior pituitary extract present in the hormone preparation. Markson (1949) administered corticotrophin for several months and found that the improvement in rheumatoid arthritis was maintained during the whole period of treatment. The patients relapsed, however, when treatment was discontinued for three days. The prolonged administration of ACTH may produce toxic effects, including Cushing's syndrome, and the patient may also become refractory to the drug, perhaps because of the formation of antibodies (Elkinton *et al.*, 1949).

Corticotrophin has also been used successfully in the treatment of rheumatic fever, of disseminated lupus erythematosus and of gouty arthritis ; in the last eondition the treatment also produeed a large inerease in the urie acid exeretion together with a fall in the serum uric acid level (Thorn and Bayles, 1949). It has also been used in a variety of allergic conditions and striking effeets have been obtained in a number of patients in the control of chronic asthma. Not only was there relief of symptoms but also objective changes in the expiratory phase of respiration and in the condition of the nasopharynx (Bordley *et al.*, 1949).

Work is in progress on the mechanism which controls the seeretion of corticotrophin by the pituitary and its relation to

adrenal cortical secretion. The paper by Thorn and Bayles mentioned above should be consulted for the evidence published up to the present. It would appear that anterior hypothalamic centres form an essential link in the activation of pituitary corticotrophin secretion in conditions of stress or following the administration of adrenaline.

Obviously much further work with these preparations remains to be done before we can assess their value in rheumatic and other conditions. Other steroids of the adrenal cortex are also being investigated. Unfortunately these substances are difficult to prepare. Merck states that no supplies of cortisone are expected for treatment or additional research until 1950, and even then the supplies will be small. At present cortisone is made by partial synthesis from desoxycholic acid by a lengthy method which involves 37 intermediate steps. It has recently been found that it can more easily be prepared from sarmentogenin, a steroid with a hydroxyl group in the 11-position, which can be extracted from the seeds of a tropical plant of the strophanthus genus (S. sarmentosus). This plant could easily be cultivated on a large scale, and thus large amounts of cortisone would be made available (Annotation, 1949), but it has been estimated that at least 20 years will be required to solve the botanical problems involved in its commercial growth (*Lancet*, 1950).

As for adrenocorticotrophin, it seems highly unlikely that any large amounts will be available from natural sources, since this substance is prepared from pituitaries of which the supply is limited. There is, however, good evidence from the work of Morris and of Li that adrenocorticotrophic action can be produced by a moderately sized peptide with a molecular weight of about 1200, the synthesis of which appears to be within the realm of possibility, and Luft *et al.* (1949) have shown that such a peptide, obtained from the peptic digestion of pure ACTH, is effective in the treatment of rheumatoid arthritis.

A very interesting finding is that when deoxycortone, or its acetate, is perfused through isolated adrenal glands, the perfusate contains corticosterone, which has a hydroxyl group at C_{11}, i.e. a bio-oxygenation of deoxycortone at C_{11} has taken place (Hechter *et al.*, 1949). At present this does not appear to be of any practical importance, though it does suggest the possibility that a similar conversion might occur in the presence of suitable micro-organisms.

Deoxycortone and Ascorbic Acid

A new approach to the treatment of rheumatoid arthritis with steroids has been given by the results of Lewin and Wassén (1949). These investigators obtained striking relief of the condition by administering 5 mg. of deoxycortone acetate intramuscularly, followed by 1 G. of ascorbic acid intravenously or intramuscularly. This work has been repeated by a number of investigators, some of whom have confirmed it (e.g. Le Vay and Loxton, 1949; Douthwaite, 1949) while others have failed to do so (e.g. Kellgren, 1949; Spies *et al.*, 1949). From the evidence available it seems difficult to believe that the combination of deoxycortone and ascorbic acid does not produce beneficial effects in some cases, and it has indeed been reported that similar effects can be produced by giving intravenous ascorbic acid after the intramuscular injection of progesterone (2 mg.) or of testosterone (20 mg.) (Landsberg, 1950). Le Vay and Loxton (1950) have obtained similar responses in various other joint conditions, e.g. monoarticular infective arthritis and traumatic conditions, and have also produced evidence that the actions of deoxycortone and ascorbic acid are exerted peripherally.

Experimental Approach

It would be a great advantage if experimental methods could be devised with which the possible value of remedies in rheumatoid arthritis could be investigated. Following the finding of Thorn *et al.* (1948) that in normal subjects the administration of ACTH causes a fall in the circulating eosinophils, Spies and Stone (1949) obtained similar results in patients with chronic eosinophilia of unknown origin. They then tested the action of a number of steroids (other than cortisone) in these patients and found that none of them produced any change in the blood eosinophils. Subsequently they tested these steroids in cases of rheumatoid arthritis and found them to be of no value (Spies and Stone, 1950). The suggestion is that substances of value in rheumatoid arthritis would cause a fall in the blood eosinophils and could thus be discovered.

Selye (1949) has elaborated a method with which an acute arthritis and periarthritis can be produced in rats by the local injection of formaldehyde into the vicinity of joints. If large doses of formaldehyde are given, the acute stage is followed by a very

prolonged chronic arthritis and periarthritis. This "formalin-arthritis" can be almost completely antagonized by the administration of cortisone or ACTH and may thus prove of value in the search for substances which produce similar effects. Using this technique Brownlee (1950) has shown that a combination of deoxycortone and ascorbic acid will protect rats against formaldehyde arthritis. Similar results have been obtained by Buttle (personal communication) in periarthritis in mice, though he also found that ACTH was much more effective than deoxycortone and ascorbic acid.

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SYNTHETIC ŒSTROGENS

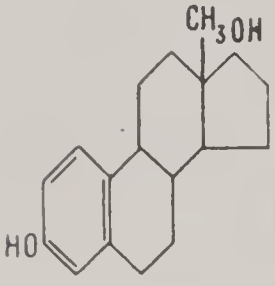
The main advantage of synthetic œstrogens over the naturally occurring compounds is that they are also active when given orally. Ethinyl œstradiol, a synthetic derivative of œstradiol, is also orally active. It was first described in 1938 by Inhoffen and Hohlweg but has only been extensively used during the last few years.

The main synthetic œstrogens are stilbœstrol, dienœstrol, hexœstrol and triphenyl ethylene derivatives, of which DBE is the most interesting. Benzœstrol, which has a structure reminiscent of hexœstrol, was prepared by Stuart and Tallmann in 1942. The older synthetic œstrogens, with the exception of ethinyl œstradiol, require no discussion here. Solmssen (1945) gives a masterly review of the synthetic œstrogens discovered up to that year.

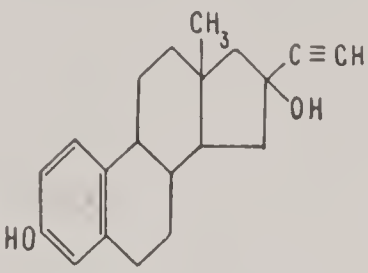
Many additional compounds have been prepared since then. The most interesting and active ones are doisynolic acid and its derivatives, and derivatives of allenolic acid. These two compounds were so named after Doisy and Allen respectively. Doisynolic acid was prepared by Miescher (1944) and has the general steroid structure, with the fourth ring opened up. It has been fully synthesized by Anner and Miescher (1947). Allenolic acid and its derivatives were prepared by Horeau and Jacques (1947). The formula of this substance can be written as shown on p. 120 and

its general resemblance to œstradiol can be seen, though the opening of the rings has been carried one stage further than in doisynolic acid.

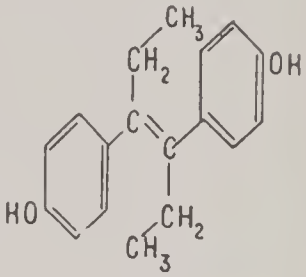
These new œstrogens are being tested clinically but there is, as yet, no indication that they possess any advantages over substances like stilbœstrol and dienœstrol.



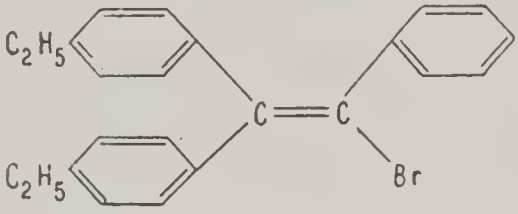
Œstradiol



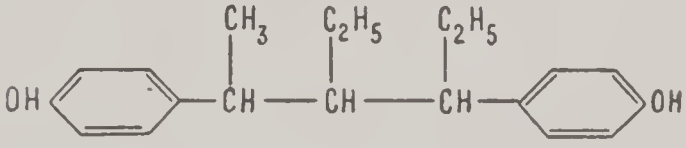
Ethinyl Œstradiol



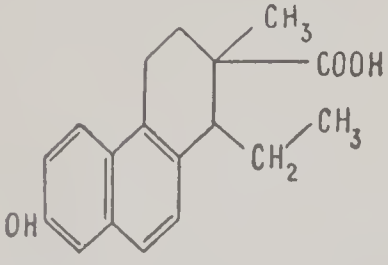
Stilbœstrol



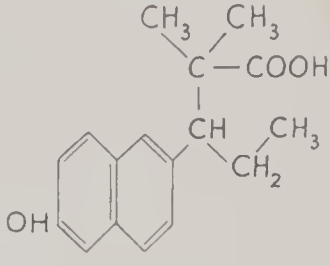
D B E



Benzœstrol



Bisdehydro doisynolic acid



Dimethyl ethyl allenolic acid

Clinical Use of Ethinyl Œstradiol

This is probably the most active oral œstrogen in the human subject, and its properties are reviewed by Thompson (1948).

At first the doses used clinically were too high and frequently produced toxic reactions, and it is now recognized that lower doses are effective and rarely produce undesirable side effects. When the amount given per day is above 0.1 mg. nausea, vomiting, headaches, congestive vaginal bleeding, and tingling and sensitivity of the breasts and nipples are frequently seen in women. In men gastro-intestinal disturbances are usually absent even with high doses, but there is some tendency to gynecomastia. Toxic effects in women are rarely seen with doses of 30 μg. per day or less. During pregnancy and shortly after term women appear to tolerate large doses well. For example Kurzrok (1948) administered 0.3 mg. of ethinyl œstradiol every two hours for periods up to 72 hours in order to produce evacuation of the products of conception in cases of missed abortion. The results were good and no untoward reactions were observed. Jeffcoate *et al.* (1948) investigated the value of the drug in the inhibition of lactation. Satisfactory effects were obtained with total doses of 0.75 to 1.3 mg. spread over seven days. No ill effects were noted. These authors found that, by this test, ethinyl œstradiol is at least 50 times as active as stilbœstrol. The relative activities of some other synthetic œstrogens are discussed by Bishop *et al.* (1948).

It is not intended to review here the uses of œstrogens (these are discussed in the book edited by Greene, 1948). It seems clear that ethinyl œstradiol can be used for all purposes for which œstrogen treatment is necessary. The dosage has to be carefully considered in every case. It is likely that in the treatment of menopausal symptoms amounts of 30 μg. per day, or less, may be adequate, and that unless this is recognized toxic effects may be unnecessarily produced.

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CHAPTER 6

THE THYROID

The thyroid was the first endocrine gland to be discovered and its active principle, thyroxine, was identified by Kendall and synthesized by Harington and Barger. Recent work with radio iodine (see Chap. 16) has thrown further light on the production of thyroxine in the body. (See review by Rawson and MacArthur, 1947.) Important advances have also been made in the synthesis and clinical use of antithyroid substances.

The Synthesis of Thyroxine

When a small amount of iodine is administered, most of it is rapidly taken up by the thyroid gland and by means of the autoradiograph technique (see Chap. 16) it can be shown that the iodine is deposited in the colloid. The experiments of Leblond and Gross (1948) suggest that the circulating iodide is continuously being bound to protein in the cytoplasm of the thyroid cells, and that the thyroglobulin so formed is then deposited in the colloid of the thyroid follicles. The circulating thyroid hormone, according to Taurog and Chaikoff (1948), consists of thyroxine loosely attached to plasma protein (probably mainly α-globulin and albumen, and to a less extent, β-globulin). Small amounts of iodine are also taken up by other tissues, especially the lungs and kidneys (Ariel *et al.*, 1941).

The administration of thyrotropic hormone causes hypertrophy and hyperplasia of the thyroid gland and also increases its capacity to take up iodine. Thus Stanley and Astwood (1949) found that in normal human subjects the administration of thyrotropin was followed, 8 hours later, by an increase in the uptake of radio iodine by the thyroid gland ; this increase attained a maximum in 24–48 hours and returned to the pre-injection levels in 4–5 days. It is interesting that iodine stored in the thyroid is also more rapidly released, probably as thyroxine, under the influence of thyrotropic hormone. Hence this hormone appears to control both the storage and the discharge of iodine from the thyroid gland (Rawson and

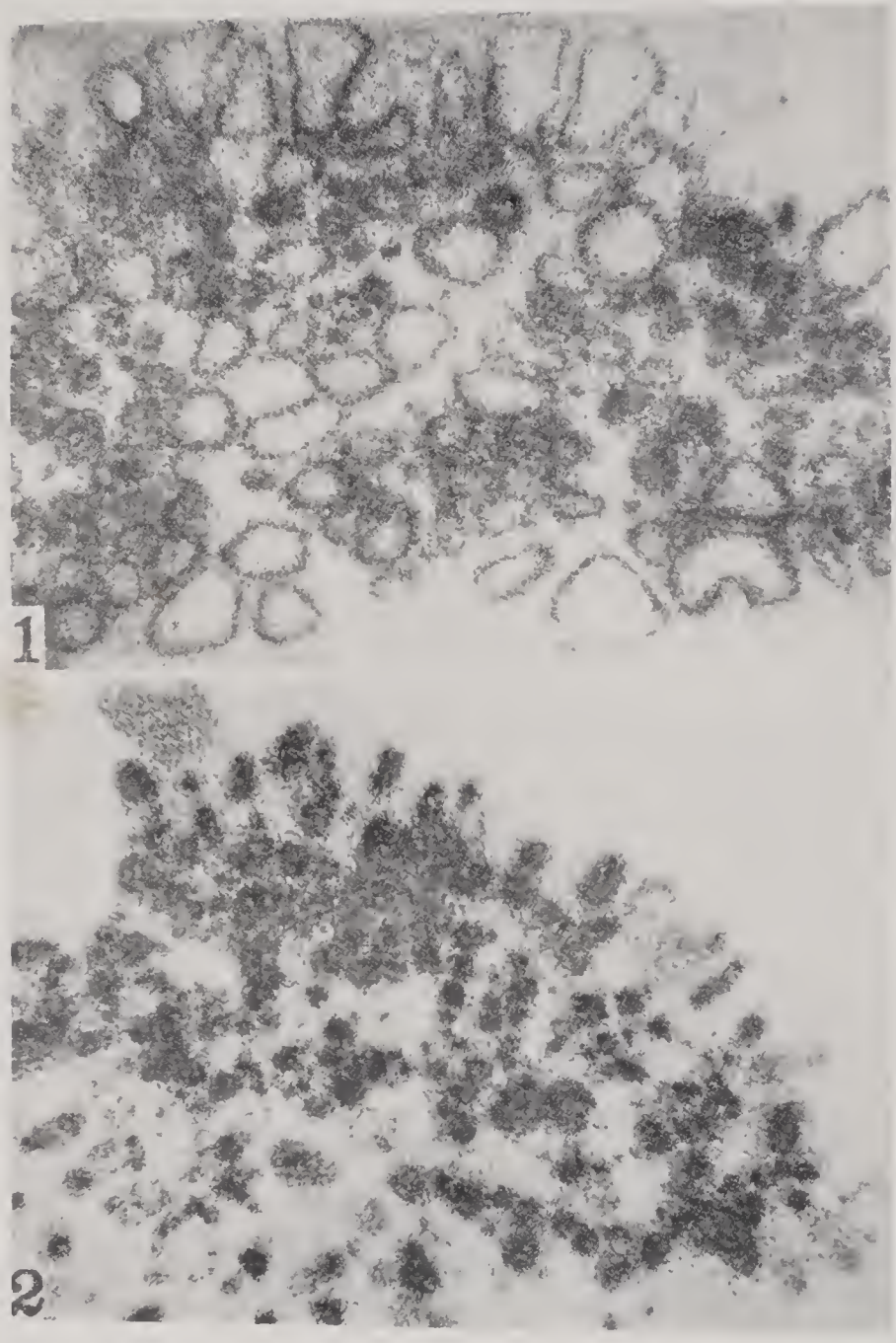


FIG. 10

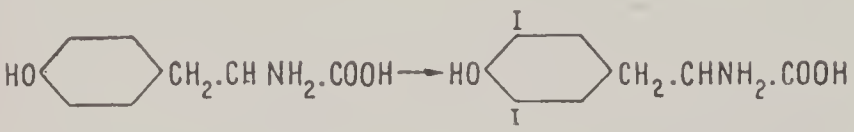
Showing the deposition of iodine in thyroid follicles.

1. Unstained autoradiograph of thyroid from rat killed one hour after injection of radio iodine. Most follicles appear as rings because of the deposition of photographic granules at the level of the epithelium. ×65.
2. Similar picture from rat killed 24 hours after injection of radio iodine. Practically all follicles appear as dots, because of the deposition of photographic granules over the colloid. ×65.

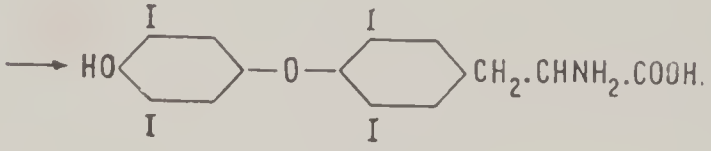
(After Leblond and Gross, 1948.)

[*To face page* 122.

MacArthur, 1947). In hypophysectomized animals, in which no thyrotropic hormone is of course produced, less iodine is stored by the thyroid, though still much more than in other tissues (Leblond and Süe, 1941).



Tyrosine Diiodotyrosine



Thyroxine

Iodine is taken up by the thyroid gland much better in hyperthyroid than in hypothyroid patients (Keating *et al.*, 1947), or than in normal individuals (Stanley and Astwood, 1948); this was shown in experiments with radio iodine. Studies with radio iodine also support the view that thyroxine is formed from tyrosine which is first iodinated to monoiodotyrosine (Fink and Fink, 1948; Taurog *et al.*, 1949), then to diiodotyrosine, and finally converted to the thyroid principle. This series of changes can be followed not only in intact thyroid glands but also in slices of the organ surviving *in vitro*. When iodine is given to animals or man much of it is fixed by the thyroid, in which it is found in the form of inorganic iodine, diiodotyrosine and thyroxine. After some time a greater proportion of the administered iodine is found as thyroxine, which has been formed from diiodotyrosine (Mann *et al.*, 1942). The administration of an excessive amount of iodine to rats, however, inhibits the formation of organically bound iodine in the thyroid gland, by inhibiting both the incorporation of iodine into diiodotyrosine and the conversion of this compound to thyroxine. This too has been demonstrated by the administration of radio iodine (Wolff and Chaikoff, 1948). It is possible that this may explain the remarkable action of iodine in inhibiting the excessive thyroid activity in exophthalmic goitre.

Of very great interest is the finding that thyroxine can be formed not only in the thyroid but also by tissues outside this gland, an important discovery also achieved by the use of radio iodine (Morton *et al.*, 1943). These investigators administered iodine to completely thyroidectomized rats, and 24 to 90 hours later found that as much as 30 per cent of this iodine was present in organic combination. By careful chemical analysis they were able to show that this organic material contained both diiodotyrosine and

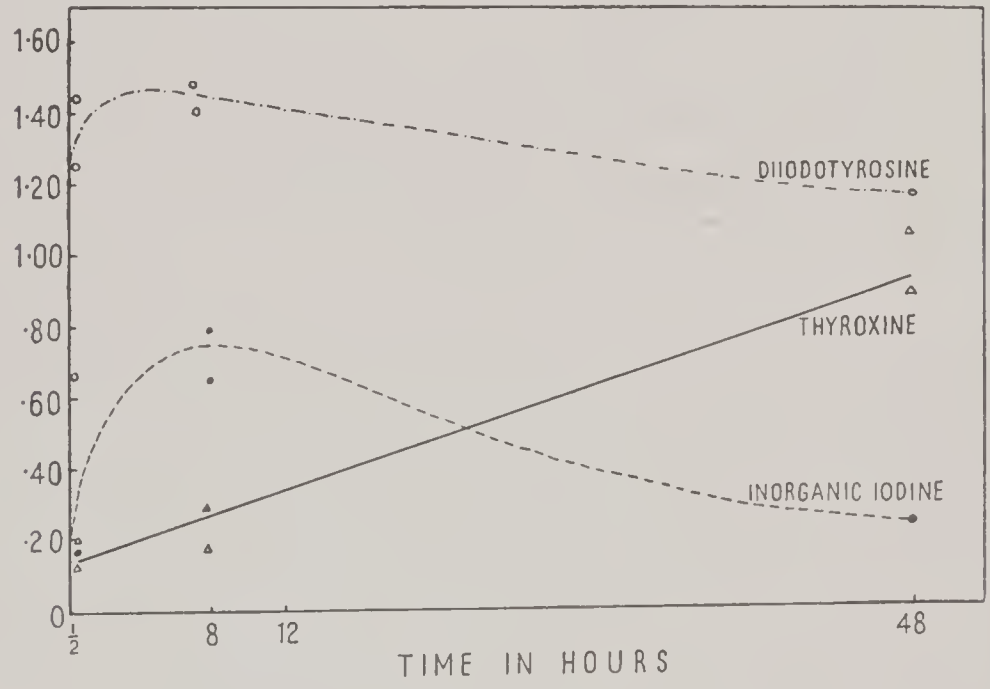


FIG. 11

Showing the relative specific radio-activities in the thyroid gland of dogs at various periods after the injection of radioactive iodine. (After Mann *et al.*, 1942.)

thyroxine. This finding, which is now being further investigated, naturally raises the question whether the metabolic activities which occur in the absence of the thyroid are not, to some extent, controlled by thyroxine formed outside the thyroid gland.

Harington (1947) believes that it can now be concluded that the essential step in the biosynthesis of thyroxine is the liberation of iodine from iodide by an enzymic oxidizing system. If this occurs in living tissue, in which tyrosine is present, this amino acid

is first converted to diiodotyrosine and the iodine then oxidizes the latter to thyroxine.

Thyroxine is continually being formed by the thyroid, and it is rapidly excreted into the intestine by the liver. This has been shown by injecting rats with thyroxine containing radio iodine and following its fate in the body (Gross and Leblond, 1947).

Iodinated Proteins

A recent development, which may prove of great practical importance, is the finding that by the iodination of tyrosine rich proteins, such as casein, a product is obtained which contains thyroxine and exhibits the biological activity of thyroid hormone. This was first demonstrated by Ludwig and Mutzenbecher (1939), and the subject is reviewed by Reineke (1946), who describes the extensive chemical work that has been done to determine the optimum conditions for iodination. By careful control of the conditions it is now possible to produce consistently preparations containing 3 to 4 per cent of thyroxine, as determined by either chemical analysis or biological assay (Reineke *et al.*, 1945). It still remains to be proved definitely that all the biological activity in the material is due to its thyroxine content.

These artificial preparations are active when administered either orally or parenterally but hardly any clinical work has been done with them, although this seems desirable now that highly active preparations are available. Successful responses in myxœdema and cretinism were described in 1939 by Lerman and Salter, and more recently De Gennes and Deltour (1948) have described the value of iodocaseins containing up to 1.7 per cent of thyroxine in cases of hypothyroidism. These authors point out that these substances have a stronger effect on myxœdema than thyroid preparations, without causing unpleasant side-effects, such as palpitations, tachycardia, diarrhoea and nervous irritability. Hamilton and his co-workers (quoted by Kelsey *et al.*, 1949) describe the use of iodocasein labelled with radioactive iodine in the treatment of a case of myxœdema.

A fair amount of work has been done on the possible use of iodinated proteins in farm animals. Thus it has been shown that the feeding of carefully regulated doses of such substances to cows in declining lactation causes an increase in the milk yield, and the fat content of the milk is appreciably increased. The milk does not

contain any quantities of thyroxine detectable by biological methods (Reineke, 1946 ; Blaxter, 1945). A similar effect can be produced by the feeding of synthetic *l*-thyroxine to cows (Bailey *et al.*, 1949). There is also evidence that the summer decline in egg production in hens can be prevented to a great extent by feeding them with optimum amounts of iodinated protein, but this requires confirmation (Reineke, 1946).

Antithyroid Substances

Up to a few years ago the only methods available for the treatment of hyperthyroidism were either removal of part of the thyroid gland or the administration of iodine. The latter, however, produces only temporary benefits, and is used only as a preliminary to operation. The discovery of substances which influence the functional activity of the thyroid therefore represents an important advance, though their exact place in therapeutics is still a matter of controversy. By the use of radio iodine it has been possible to elucidate, to some extent, the mechanism by which various antithyroid substances produce their effect.

Discovery

The discovery of antithyroid substances really dates back to 1928, when Chesney *et al.* (1928) found that in their colony of rabbits, which were being fed mainly on cabbage, many animals developed hyperplasia of the thyroid gland ; the significance of this was at first not realized ; later similar effects were also found in animals fed with other plants of the Brassica Genus. These plants are known to contain nitrile derivatives, and shortly afterwards Marine and his co-workers (1932) found that the administration of acetonitrile to rabbits caused striking hyperplasia of the gland.

For a period no further progress was made but in the early 1940's several new goitrogenic substances were discovered, including to begin with, sulphaguanidine and later, allylthiourea and thiourea (Mackenzie *et al.*, 1941 ; Richter and Clisby, 1942 ; Kennedy, 1942). Astwood and his co-workers confirmed and extended these observations (Astwood *et al.*, 1943) and showed that these substances interfered with the synthesis of the thyroid hormones in the thyroid gland, but did not antagonize the action of the thyroid hormone in tissues. Astwood investigated a large number

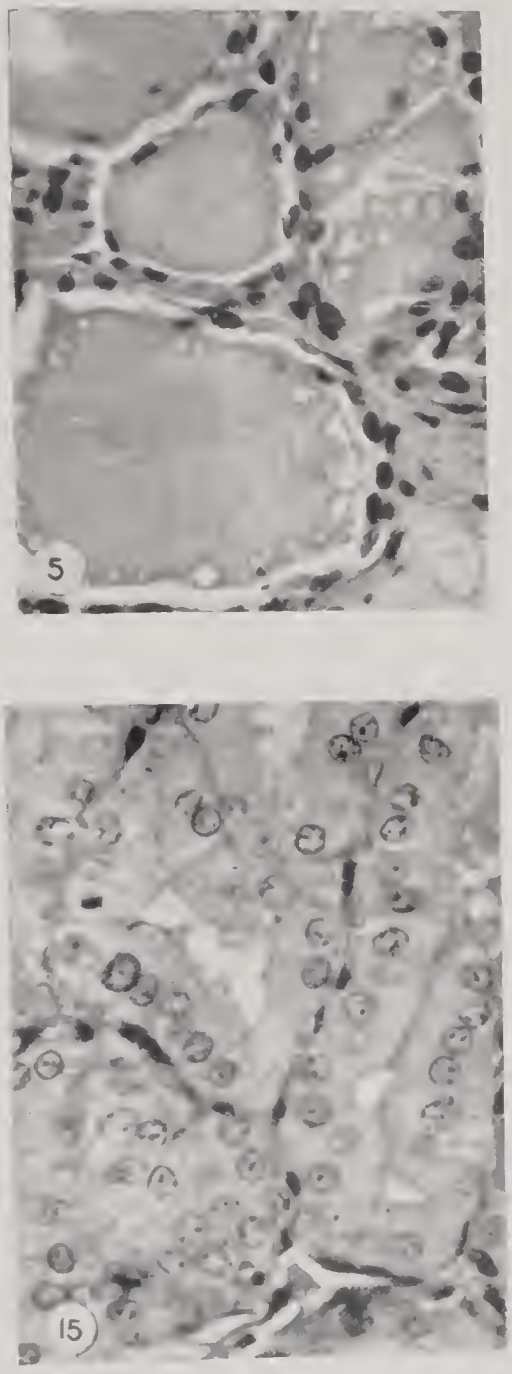


FIG. 12

Showing the effect of treatment with anti-thyroid substance on the height of the thyroid epithelium.

5. Thyroid of young rat on stock diet. × 600.

15. Thyroid of young rat fed 0.66 per cent thiourea for 21 days. × 600.

Note absence of colloid and increase in height of epithelial cells.

(After Mackenzie and Mackenzie, 1943.)

To face page 127.]

of other substances and was the first to apply his results to the treatment of human thyrotoxicosis (Astwood, 1943) ; he observed that the effect came on after a latent period of one to two weeks. At present thiouracil derivatives are the substances most commonly used, but work is actively proceeding to produce less toxic and more active substances.

When these substances are given to animals they produce the characteristic signs of thyroid hypofunction, including a gradual reduction in the basal metabolic rate, a decreased food intake and an impairment of growth and development. The thyroid gland becomes enlarged and shows colloid loss, increased vascularity and increase in the height of the acinar epithelium.

Mechanism of Action of Antithyroid Substances

It is clear that antithyroid substances like thiourea and thiouracil produce their effect by inhibiting the conversion of iodine to diiodotyrosine and thyroxine. The taking up of inorganic iodine is apparently not affected, but only its conversion to organic iodine (Taurog *et al.*, 1947 ; Vanderlaan and Vanderlaan, 1947 ; Stanley and Astwood, 1948). The effect of these goitrogens on the formation of thyroxine has been demonstrated both *in vitro* and *in vivo* (Franklin *et al.*, 1944). The *in vivo* experiments were performed on rats : the animals were fed on thiouracil for a week, injected with radio iodine and killed 48 hours later. The thyroids contained about half the amount of radio-thyroxine and about one third the amount of radio-diiodotyrosine found in normal glands.

The effect on the uptake of radio iodine by the thyroid has been used as a method of estimating the relative efficiency of various goitrogenic substances. Marked effects were produced both in rats and chicks by benzyl thiouracil, propyl thiouracil and thiouracil (McGinty and Rawson, 1947). The method has proved particularly useful in evaluating the effect of these drugs in man. Thus Stanley and Astwood (1947) determined the rate of accumulation of iodine by the thyroid of normal subjects by serial counts with a Geiger-Muller counter placed on the thyroid region, and showed that the uptake was reduced by a number of antithyroid drugs. They found a close correlation between the results so obtained and those recorded in clinical trials.

An important feature of the action of these substances in animals

is the enlargement of the thyroid gland which is produced. The mechanism of this effect is probably as follows: Normally, the secretory activity of the thyroid is maintained by the thyrotropic hormone of the anterior pituitary gland. The output of this hormone, however, is itself regulated by the concentration of thyroid hormone in the circulation. If the formation of thyroxine is inhibited by a substance like thiouracil, then the inhibition of the anterior pituitary will be removed and the output of thyrotropic hormone will be increased; this in turn will produce a glandular hyperplasia of the thyroid. There is much experimental evidence in support of this view, including the finding that thyroxine inhibits the secretion of thyrotropic hormone; in addition thyroxine also depresses the response of the thyroid to thyrotropin (Cortell and Rawson, 1944).

The mode of action of some other antithyroid substances has also been investigated. The effects of potassium thiocyanate, which has been used in the treatment of hypertension, are of some interest. The results obtained by different investigators are not all in agreement, but suggest on the whole that the drug inhibits the uptake of iodine by the thyroid rather than its conversion to thyroxine (see Kelsey *et al.*, 1949).

Absorption, Distribution and Excretion of Antithyroid Drugs

Thiouracil is rapidly absorbed from the intestine both in animal) and man. Thus it is present in the blood (about 2 mg. per 100 ml.s some 15 minutes after its administration to a fasting man (Williams and Kay, 1944; Williams *et al.*, 1944). The drug is distributed throughout the body fluids including the cerebrospinal fluid, but its concentration in the red cells is several times higher than in the plasma. Milk contains about three times as much as the blood, and the drug passes through the placenta producing significant concentrations in the fœtus. It is rapidly excreted in the urine, the maximum excretion occurring during the second hour after ingestion, and it is almost complete in about 24 hours. Some of the drug is destroyed in the body. McGinty *et al.* (1948) have shown that in man about 40 per cent of thiouracil and methylthiouracil are excreted in the urine within 24 hours. Not more than 10 per cent of propylthiouracil and none of the benzyl compound are excreted in the free form in the same period. These

results show that benzylthiouracil and, to a lesser extent, propylthiouracil are conjugated in the body and eliminated as an acid hydrolysable complex of unknown nature.

Thiouracil derivatives accumulate in the thyroid gland, but there is no definite correlation between the amount of drug in the thyroid and its antithyroid activity. In a patient treated with thiouracil who died suddenly, the drug was found to be concentrated in the adrenals, ovaries, spleen and liver (Williams and Kay, 1947).

Clinical Use

There is still much controversy about the place of thiouracil and its derivatives in the treatment of hyperthyroidism. The use of these drugs is discussed by Himsworth (1948). The disease can easily be controlled, but toxic effects are not uncommon (10–20 per cent of cases), are sometimes serious, and may even be fatal. Hence some authorities believe that these drugs should be used only to prepare patients for operation. Others believe that, if proper precautions are taken, they are suitable for full treatment of the disease, and point out that the operative risk is by no means negligible, especially in unskilled hands.

Administration of the drug produces no great change in the size of the thyroid, but symptoms are generally relieved. The metabolic rate falls, usually to normal, and the body weight increases. Tachycardia disappears slowly but there is usually little effect on the exophthalmos. In contrast to iodine therapy there is no tendency to "escape" during continued administration. The auricular fibrillation in thyrotoxicosis can frequently be converted to normal sinus rhythm by administration of thiouracil compounds (see Goodwin, 1949).

Dosage

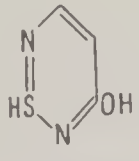
The dose has to be carefully considered. At first large doses of thiouracil, i.e. 1.0 gram per day or more, were used and toxic effects were common. It was soon found, however, that beyond a certain point, increase in dose does not increase the effectiveness of the drug and nowadays the treatment is usually started with smaller doses, e.g. 200 mg. of methyl thiouracil per day, and this is decreased to a daily maintenance dose of 25, 50 or at most 100 mg. per day (Himsworth, 1948).

Toxic Effects

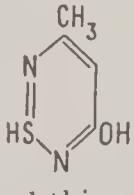
The treatment has to be continued for a long time, several months or more, and even then relapses are frequent and occur at various periods after the end of treatment. The patient has to be watched carefully as toxic reactions, many of them allergic in type, may occur, sometimes very suddenly. Mild toxic effects such as fever, urticaria, conjunctivitis, nausea, etc., are controlled when the drug is withdrawn or the dosage reduced. Serious reactions involve the blood forming organs and may include agranulocytosis in 2 per cent; 25 per cent of these cases are fatal, i.e. 0.5 per cent of the total. Neutropenia does not necessarily lead to agranulocytosis which may, however, occur without any warning neutropenia. Every patient should be aware of these possibilities, and on the appearance of fever or of a sore throat, thiouracil therapy should be stopped, the doctor should be informed and the blood examined. If granulocytes are few or absent, large doses of penicillin should be given.

The value of antithyroid drugs in preparation for surgery has been firmly established. Treatment should be continued until the thyroid function is normal and in some patients it may take months before operation can be safely undertaken. The treatment, however, leaves the thyroid in a hyperplastic and vascular condition which makes the operation technically difficult. This can fortunately be overcome by the simultaneous administration of iodine and it is now generally recommended that iodine, in the form of Lugol's solution, be given for some three weeks before the operation, except in patients in crisis or near crisis states, who need immediate administration of iodine (Bartels, 1948; Lahey and Bartels, 1947; Grauer *et al.*, 1948). An iodinated compound, 5-iodo-2-thiouracil, possesses the actions both of iodine and of thiouracil on the thyroid, and it has been suggested that it might be suitable for the preparation of thyrotoxic patients for thyroidectomy (Williams *et al.*, 1949).

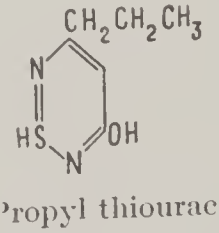
Value of Different Compounds



Thiouracil



Methyl thiouracil



Propyl thiouracil

A number of other compounds have been made and tested in the hope of finding one which will be more potent and less liable to produce serious toxic effects. Williams (1947) gives details of a clinical comparison of various substances. Some results suggest that methylthiouracil (Barfred, 1947 ; Meulengracht and Kjerulf-Jensen, 1948) and *n*-propyl-thiouracil (Astwood and Vanderlaan, 1945) are more satisfactory. Thus McCullagh *et al.* (1947) used the propyl compound in 215 cases for an average of eight months, and on some cases for periods up to 14 months, with no agranulocytosis (see Chart II in this paper). The results of Bartels (1948) too, suggest that propylthiouracil is less toxic than thiouracil or its methyl derivative. According to Himsworth (1948), however, there is little to choose between thiouracil, methylthiouracil and propylthiouracil.

There are reports that vitamin A, in massive doses (up to 400,000 i.u. per day), controls exophthalmic goitre but this needs further investigation (Simkins, 1947). The possible mechanism of this effect is discussed by Sadhu and Brody (1947).

Thiouracil and its derivatives have been used in the treatment of various other conditions including angina pectoris (Raab, 1945 ; Schoenewald, 1948) and in cases of congestive heart failure not responding to other forms of treatment (Sharpey-Schafer, 1946).

Radio Iodine

Metabolism

The use of radio iodine in thyroid disease is reviewed by Kelsey *et al.* (1949), who also discuss the fate of radio iodine in the body. It is rapidly absorbed in the stomach and can be detected in the human hand within 3–6 minutes. About 75 per cent of the drug is absorbed in the gastro-intestinal tract in one hour and absorption is apparently complete in three hours. There is evidence that radio iodine is distributed throughout the extracellular fluids. Some of the dose absorbed is probably secreted back into the stomach, since the gastric glands in man have been shown to secrete radio iodine in high concentrations when it is injected intravenously. The concentration in the blood rises to a maximum within an hour after ingestion and then falls gradually. The fall is more rapid in hyperthyroidism and slower than normal in myxœdematous patients. A large part of the injected dose is, as already mentioned, collected by the thyroid but other tissues, particularly

the ovaries, pituitary, lungs, kidneys, liver and muscles also take up small amounts of radio iodine. The "clearance" of radio iodine by the thyroid has been determined by Myant *et al.* (1949) by doing counts over the region of the thyroid, from which the iodine content of the gland can be estimated. Blood samples are removed and their iodine content determined. From these figures the rate of clearance of iodine from the blood can be calculated. In normal subjects about 16 ml. of plasma are cleared of iodide per minute by the thyroid. In untreated thyrotoxic patients the clearance rate averaged 486 ml. per minute, ranging from 200–1400 ml. per minute. The renal clearance rate for plasma radio iodide averaged about 30 ml. per minute in both normal and thyrotoxic subjects. The authors believe that the thyroid clearance test is a more sensitive and direct measure of iodine-collecting activity than previous tests based on radio iodine, and may prove of greater value than the basal metabolic rate in diagnosing minor degrees of thyrotoxicosis.

In normal subjects a large part of an ingested dose, i.e. about 70 per cent, is excreted in the urine within 48 hours, after which time only minute quantities are excreted. In myxœdema a higher proportion of the dose (up to 90 per cent) is recovered in the urine, while in hyperthyroidism smaller or much smaller amounts are usually excreted by the kidneys (Keating *et al.*, 1947). The excretion of radio iodine is being investigated as a method of diagnosis in thyroid disease. A dose of not more than 10 microcuries given orally is sufficient and can be safely used as a routine procedure. The method provides a satisfactory diagnostic aid in cases of doubtful thyrotoxicosis. Impairment of the renal function, however, makes interpretation of the excretion curve difficult and may invalidate the result (Mason and Oliver, 1949 ; Arnott *et al.*, 1949).

Therapeutic Use

Radio iodine has been used in the treatment of *hyperthyroidism* and of *cancer of the thyroid*. As the substance is stored in the thyroid the radiation emitted would be expected to inhibit the functional activity of the gland, and thus to control the growth of malignant tissue as does exposure to X-rays. In dogs and rabbits it has indeed been possible to produce almost complete destruction of the thyroid with radio iodine without evidence of damage to

other tissues of the body (Hamilton and Lawrence, 1942). Mixtures of the 12 hour and 8 day isotopes, or the 8 day isotope alone, have been administered in cases of hyperthyroidism and a number of them have responded well (Chapman and Evans, 1946; Soley *et al.*, 1949). Prinzmetal *et al.* (1949) have obtained favourable results in cases of severe diffuse toxic goitre with complications. Further

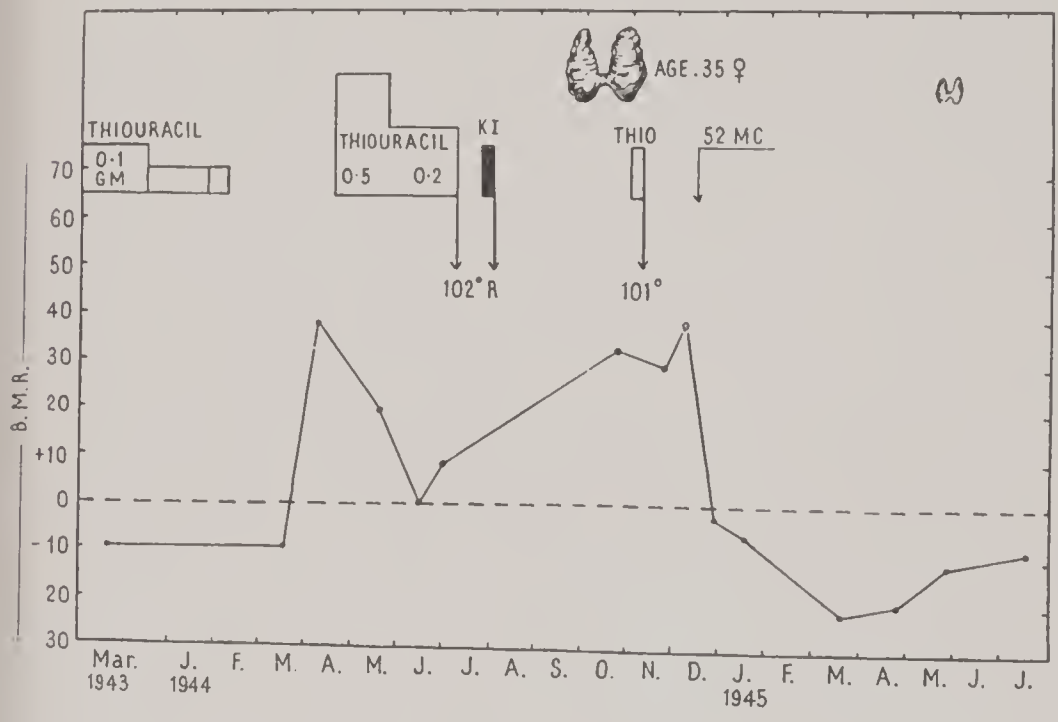


FIG. 13

Showing the effect of a single dose of radioactive iodine (52 MC) in a patient with hyperthyroidism who became hypersensitive to thiouracil and iodine. Thiouracil produced a rise in temperature and potassium iodide produced extensive dermatitis.

(After Chapman and Evans, 1946.)

work is necessary, especially to determine what doses are safe. According to Haines *et al.* (1948), it is usually necessary to give more than 200 microcuries per gram of thyroid tissue. Favourable results have also been reported in the treatment of malignant disease of the thyroid (Seidlin *et al.*, 1946). Unfortunately many thyroid tumours do not take up iodine and hence this treatment can be of value only in a minority of cases. There is evidence,

however, that the uptake of iodine by metastatic thyroid tumours can be favourably influenced by thyroidectomy or by injections of thyrotropic hormone, which increases the avidity of thyroid tissue for iodine (Seidlin *et al.*, 1948 ; Rawson *et al.*, 1948, 1949).

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CHAPTER 7

BACTERIAL CHEMOTHERAPY

This is a field which has grown tremendously in the last fifteen years and is still expanding. It will only be possible to deal with certain aspects of recent developments within the limits of the space available. More details will be found in the volume on chemotherapy in the Recent Advances series, and the publication on "Antibiotic Substances, Their Biological and Chemical Properties" published by the Antibiotics Study Section of the National Institute of Health (U.S.A.) gives, clearly and concisely, much specialized information, as does the book by Kolmer (1947).

Penicillin

Different Forms

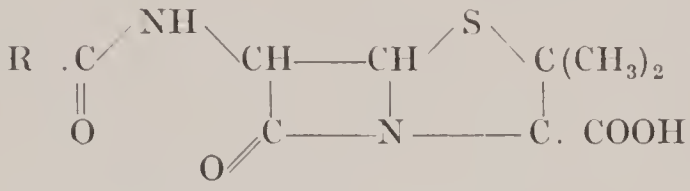
The story of the discovery of penicillin by Fleming, Florey and Chain, is now quite familiar, though it still remains a very remarkable achievement. Penicillin is obtained as a metabolic product of various moulds, notably penicillium notatum. It has been synthesized, but the yield is small and this is, at present, of no practical value (Du Vigneaud *et al.*, 1946).

The unit of penicillin is defined as the activity of 0.65μg. of the master standard as measured under standardized conditions. The need for an international unit is particularly great, since there are many forms of penicillin, which vary in their biological activity, a fact which has proved to be of practical importance. The separate pure penicillins are available only for experimental work. A pure preparation, consisting of a mixture of the various forms of penicillin, can be obtained, as can also a crystalline preparation which consists essentially of penicillin II (G). It has been suggested that the impure preparations, in common use, may in fact be more active in certain conditions, but this requires confirmation (Dunham and Rake, 1945).

The chemistry of penicillin is extensively discussed in the book edited by Clarke, Johnson and Robinson (1949), which gives details of many of the investigations performed in Britain and

U.S.A. during the war. A short account is given by Chain (1948).

All known forms of penicillin have the same general formula, differing only in one group. Their nomenclature is complicated by the fact that different names were used in Britain and U.S.A. as knowledge about them developed in the rather secret atmosphere of the war; both nomenclatures are given below :—



General formula of penicillins

Some Forms of Penicillin

(The American nomenclature is given in brackets)

	R in the above formulæ.	
Penicillin I (F)	Δ_2-pentenyl.	$CH_3.CH_2.CH=CH.CH_2—$
Dihydropenicillin I (F)	n-amyl.	$CH_3.CH_2.CH_2.CH_2.CH_2—$
Penicillin II (G)	benzyl	$C_6H_5.CH_2—$
Penicillin III (X)	p-hydroxy-benzyl	$OH.C_6H_4CH_2$
Penicillin IV (K)	n-heptyl	$CH_3CH_2CH_2CH_2CH_2CH_2CH_2—$

Activity and Metabolism

The activity of these various penicillins against different organisms differs *in vitro*, as well as *in vivo*; their activity *in vivo* is further complicated by the fact that they are metabolized and excreted at different rates. Data are given in Tables 1 and 2. Many new biosynthetic forms of penicillin have recently become available through the fascinating investigations of a group of workers of the Lilly Research Laboratories and their collaborators. They found that the addition of certain chemical precursors in the culture medium of the mould led to their incorporation into the penicillin molecule (Behrens *et al.*, 1948). Some of these new penicillins are more active than penicillin II; and some of them are less toxic (Rose *et al.*, 1949). The possibility of producing forms of penicillin active against penicillin II resistant organisms is also being investigated.

TABLE 1

Showing the relative activities in vitro *of the various forms of penicillin against* Staphylococcus Aureus *and* B. Subtilis.

Type of penicillin	Activity in units per mg. against *Staph. Aureus*	*B. Subtilis.*
II	1667	1667
I	1625	1075
dihydro I	1610	1015
III	970	1450
IV	2300	760

TABLE 2

Showing the relative activities of the different forms of penicillin against various experimental infections.

Type of infection	Therapeutic activity relative to Penicillin II (G) as 100. I	II	III	IV
Pneumococcal infections in mice	83	100	160	19
Streptococcal infections in mice	50	100	260	9
T. pallidum infections in rabbits	8	100	14	11.5

When penicillin is given parenterally, it is rapidly excreted and a variable amount, up to 70 per cent, is found unchanged in the urine. The remainder is rapidly metabolized and the inactive metabolic products are also quickly excreted : this has been shown by studies with penicillin labelled with radioactive sulphur (Rowlands *et al.*, 1948).

The rate of metabolism of the different penicillins has been studied by Eagle and Musselman (1946) who injected rabbits intramuscularly with crystalline penicillins I, II, III and IV. With penicillin IV (K) the blood level of the drug fell far more rapidly than with the other three penicillins and a lower proportion of penicillin IV than of the other three forms was excreted in the urine. Similar results were obtained in experiments in human subjects, injected with 0.6 mg. per kg. of the various forms of

penicillin. Thus a measurable blood concentration was maintained for only 70 minutes with penicillin IV and for over two hours with the other penicillins. This is obviously one of the factors accounting for the low chemotherapeutic activity of penicillin IV (K), and is of considerable clinical importance, especially in the treatment of syphilitic conditions. As the methods of preparation and purification of penicillin evolved, one stage was reached at which certain batches of the final material contained a high proportion of penicillin IV (see "The Changing Character of Commercial Penicillin," 1946). This difficulty has now been overcome and the proportion of penicillin IV in most products is now low. Much work has been done to determine which form of penicillin is best in the treatment of infection and especially syphilis, and there is good evidence for believing that penicillin II (G), or a mixed crystalline preparation containing a high proportion of penicillin II, is the preparation of choice.

The treatment of various forms of syphilis with penicillin is reviewed in the Journal of the American Medical Association (1948) by the Syphilis Study Section, National Institute of Health, and details are given about dosage and duration of treatment. The question of combining such treatment with other forms of medication (bismuth, arsenicals) is also discussed. In Britain it is generally accepted that better results are obtained with such combined treatment.

When gonorrhœa is treated with penicillin, of which only comparatively small doses are required, a simultaneously or previously acquired syphilitic infection may be masked, and not discovered until a delayed sore or secondary reactions are seen, or a positive serological reaction appears. This possibility must be borne in mind in the treatment of gonorrhœa (Fromer *et al.*, 1946), and it has been suggested that patients in whom syphilis is suspected should be watched for a period of six months. This difficulty may be overcome by using streptomycin for treating gonorrhœa in patients in whom both venereal infections may be present (Willcox, 1948).

Toxicity

Penicillin is almost non-toxic when administered to man. Allergic reactions are, however, sometimes seen, both with systemic administration and with local application, but they are

rarely serious and no deaths have been reported as due to the administration of the drug. Among the toxic reactions which do occur are various types of rashes, including very occasionally exfoliative dermatitis; nausea and vomiting; fever and serum sickness. Urticaria is the only reaction which occurs at all commonly. Some of the allergic reactions seen with penicillin are very similar to those observed in fungus diseases of the skin and it has been suggested that a fungus infection may actually sensitize a patient to penicillin (Peck *et al.*, 1948).

Impure preparations of penicillin tend to produce toxic effects when given intrathecally, but pure penicillin is well tolerated. Normally, 12,000 units will maintain a bacteriostatic level in the cerebrospinal fluid for 24 hours, but occasionally, e.g. in the early stages of pyogenic meningitis, this dose may be given every 12 hours. Larger doses, of 50,000 units or more, may cause severe meningeal reactions and should not be used. Intrathecal penicillin, if continued for some weeks, may cause depression of the ankle-jerks, suggesting damage to the cauda equina, but this change is reversible. Penicillin is usually introduced into the cerebrospinal fluid by the lumbar route, but if necessary it should be injected into the cisterna, or even into the lateral ventricles (Cairns, 1947). It should be noted that when large doses of penicillin are given intramuscularly, chemotherapeutic concentrations are quite frequently produced in the cerebro-spinal fluid (Redfearn *et al.*, 1949).

The retina is particularly sensitive to the toxic action of various drugs and it has been found that even pure crystalline penicillin II (G) is not entirely free from toxic effects when introduced into the vitreous. It has been shown that of the five forms of penicillin tested, penicillin II was the least toxic to the retinal elements (Gardiner *et al.*, 1948).

Blood Concentration

As penicillin has become more readily available the tendency has been to increase the dose, and at present the drug is frequently given in doses of 50,000 units intramuscularly every three hours, or in less serious conditions 100,000 units twice daily. Increasing use is being made of depot preparations, such as procaine penicillin, which have a prolonged effect and can therefore be given less frequently. Much work has been done on the fate of the drug in the body. After absorption, penicillin is distributed throughout

the body fluids but, owing to its rapid inactivation and excretion, the injections have to be frequently repeated in order to maintain a chemotherapeutic concentration in the blood and tissue fluids. Some of the penicillin combines with the serum proteins to form a complex. It was at first suggested that this complex, unlike that which is formed between sulphonamides and serum proteins, is chemotherapeutically active (Chow and McKee, 1945); but later evidence seems to show that such is not the case, and that appreciable inactivation does occur by binding with the serum proteins. In particular a high proportion of penicillin IV (K) combines with proteins and this may be one of the factors accounting for the low activity of this substance *in vivo* (Tompsett *et al.*, 1947).

A number of investigators have determined the blood levels that are obtained when various amounts of the drug are given per day. Thus, for example, Loewe *et al.* (1945) found that the average blood concentration is about 1 unit per ml. for each million units of penicillin injected intramuscularly or intravenously per day.

Bacteria vary greatly in their sensitivity to penicillin. For example, streptococcus viridans found in subacute bacterial endocarditis, is much less sensitive than hæmolytic streptococci. Large doses are thus used in the treatment of subacute bacterial endocarditis, up to 20,000,000 units daily (Christie, 1948; Loewe *et al.*, 1947), and an idea of the daily dose required can be obtained from the sensitivity of the organism and the amount of penicillin needed daily to produce a certain blood concentration.

Prolongation of Action

A number of methods have been used to increase the time during which an effective concentration of the drug is produced following a single injection. The aim, of course, is to reduce the number of injections per day: with aqueous solutions even large doses have to be given at relatively frequent intervals.

Combination with Oil and Beeswax. One method which has been used extensively is to inject intramuscularly a suspension of the drug in a mixture of 95.2 per cent of peanut oil and 4.8 per cent of beeswax (Romansky and Rittman, 1945). A chemotherapeutic concentration of the drug is maintained for ten or more hours, and as a rule two injections of this mixture per day are sufficient for continuous therapeutic action. Under these circumstances the patient can frequently be treated as an outpatient. This method

has, for example, been used in the treatment of syphilis. Occasionally the injection of this preparation produces local irritation. It is rather viscous, and therefore difficult to get up the syringe.

Procaine Penicillin. It has been shown more recently that crystalline penicillin II forms a complex with procaine which is sparingly soluble in saline and in oil. When this is injected, the penicillin is slowly released and chemotherapeutic blood levels are maintained for some 24 hours (Herrell *et al.*, 1947 ; Sullivan *et al.*, 1948). No local reactions are apparently produced by such a preparation. Preliminary results show that a high proportion of cases of pneumonia are cured by a single injection of 300,000 units of procaine penicillin in oil (Boger *et al.*, 1948). The preparation should also be valuable in infants and children. In babies a dose of 100,000 units produces therapeutic blood-penicillin levels for at least 24 hours, and in the period immediately after birth for considerably longer (Wilson *et al.*, 1949).

The incorporation penicillin into a gel made by the addition of *aluminium monostearate* markedly delays its absorption, and it has been shown that when procaine penicillin is suspended in such a medium, a single injection of 300,000 units will maintain a chemotherapeutic concentration in the blood for some four days (Thomas *et al.*, 1948). With 2,000,000 units the blood level is maintained for a week after the injection (Young *et al.*, 1949). Such a preparation is also suitable for use in children and the blood levels produced by its administration have been studied by Emery *et al.* (1949). The use of procaine penicillin preparations in the treatment of syphilis, and in the prevention of the disease in patients exposed to infection, is being extensively investigated in U.S.A. The very interesting and promising results were described by Dr. J. E. Moore at a meeting of the Royal Society of Medicine and have been published in detail (Moore, 1949). It seems quite clear that a single injection of 600,000–900,000 units of procaine penicillin given within 6 days to persons exposed to infection can markedly reduce the incidence of primary and secondary syphilis. The use of procaine penicillin in the ambulant treatment of syphilis is described by Scott (1949) ; 600,000 units were given twice weekly for seven and a half or ten weeks and the author concludes that "the ambulant treatment of syphilis appears to be more successful than with arsenical bismuth regimens of comparable duration." It seems possible that the search for a *therapia magna sterilans*,

initiated by Ehrlich and so long sought for in this disease, is being achieved by the use of this penicillin preparation.

Combination with Vasoconstrictor. Another method which has been used to delay the absorption of penicillin is to combine it with a vasoconstrictor substance, such as adrenaline or ephedrine. For example, Ercoli *et al.* (1948) have found that the addition of

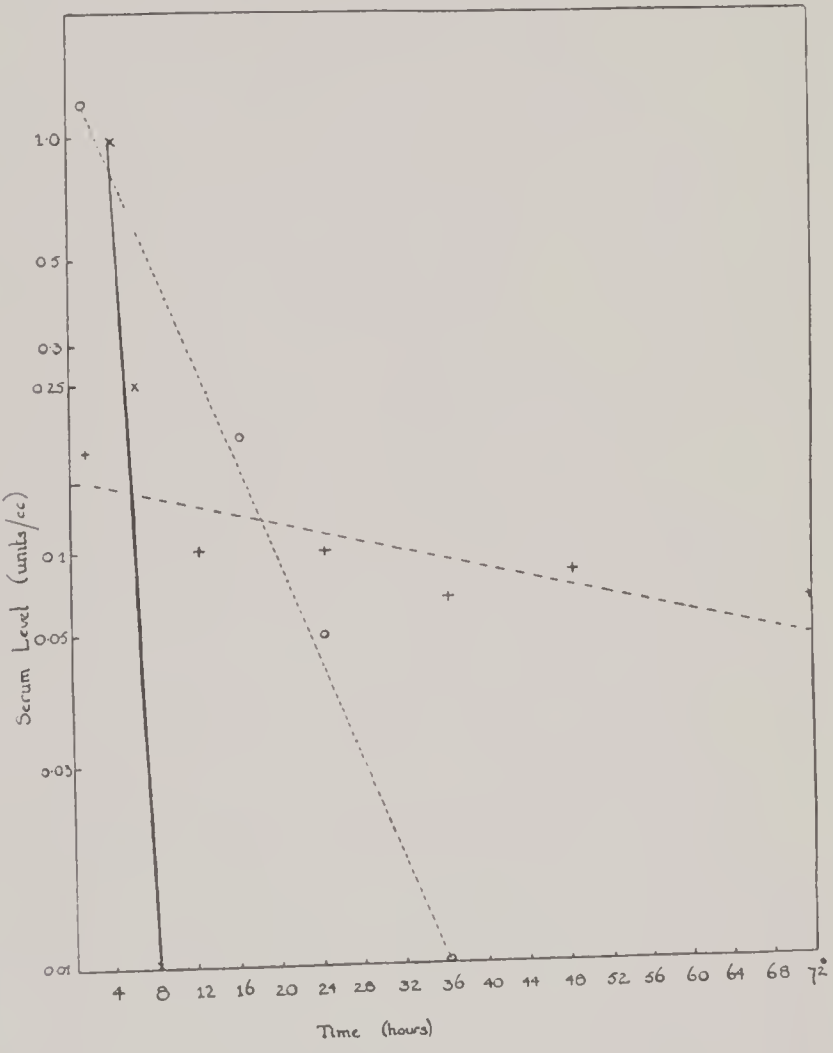


FIG. 14

Showing the serum levels of penicillin produced by the administration of 300,000 units of penicillin in different preparations.

×———× In saline (after Ercoli *et al.*, 1948).
O - - - - - O Procaine penicillin in oil.
+-·-·-+ Procaine penicillin in oil with aluminium monostearate (after Thomas *et al.*, 1948).

0.3 mg. of adrenaline to penicillin in saline and peanut oil doubles the period during which a chemotherapeutic concentration is maintained in the blood. A single dose of such a preparation has been successfully used in the treatment of gonorrhœa (Cohn and Kornblith, 1948). A preparation of penicillin II containing both ephedrine and adrenaline in gelatine dextrose has been developed by Loewe *et al.* (1947). Apart from its prolonged effect this preparation has the advantage that it is stable at room temperature for more than a year. Grunberg *et al.* (1949) have made a compound of penicillin II and l-ephedrine which exerts quantitatively the antibacterial action of penicillin and the vasoconstrictor properties of ephedrine.

Penicillin Esters. The effectiveness and duration of action of steroids can be greatly increased by using esters (e.g. œstradiol benzoate, testosterone propionate) from which the hormone is slowly released producing an even and prolonged effect. A similar method has been tried with penicillin and various esters have been made, including the methyl, ethyl, n-butyl and benzhydryl compounds (Meyer *et al.*, 1943). Experimental work on animals suggested that such esters were highly effective and could, moreover, be given orally since they were not destroyed in the stomach. Later work has shown, however, that the enzyme (esterase) necessary to decompose the ester, and thus liberate the penicillin from its combination, is not present in man : penicillin esters are thus unlikely to be of clinical value either by the systemic or oral route (Ungar, 1947).

Carinamide (Caronamide)

The prolonged maintenance of effective penicillin concentrations in blood has also been achieved by the simultaneous administration of a substance (carinamide) which interferes with the excretion of penicillin by the kidneys. Janowitz *et al.* (1948) have shown that the high blood penicillin levels so attained lead to increased diffusion through the blood-brain barrier, and hence to the production and maintenance of high penicillin concentrations in the cerebrospinal fluid. The mode of action of carinamide is discussed in Chap. 15, and a method for estimating it colorimetrically in body fluids is described by Ziegler and Sprague (1948). Boger *et al.* (1948) have investigated the relation of carinamide dosage to plasma concentration and effect on penicillin

excretion. It has been suggested that the dosage of carinamide should be so adjusted as to give a blood level of 15 mg. per 100 ml. and this can usually be achieved by giving 3 G. every 3 hours orally. Experimentally it has been found that the effectiveness of penicillin in the treatment of pneumococcal and E. Typhosa infections in mice is greatly increased by the simultaneous administration of carinamide (Verwey and Miller, 1947). The value of carinamide has also been demonstrated clinically. For example, Boger *et al.* (1947) successfully treated a case of subacute bacterial endocarditis due to a strain of streptococcus viridans, with a combination of penicillin and carinamide and demonstrated the marked elevation in the blood penicillin levels brought about by combining both these drugs. Similar results have been obtained by Stuart-Harris *et al.* (1949).

Oral Administration of Penicillin

It would be a great advantage if penicillin could be given orally instead of by repeated injections. The drug is, however, rather unstable and some of it is destroyed by the acid in the stomach, and in the intestine. When penicillin was first used orally it was combined with various antacids in order to prevent its destruction in the stomach. It was then found, however, that even with these precautions much of the penicillin was lost, and realized that the absorption of penicillin from the intestine is rather poor and irregular. There is no evidence that any of the types I, II, III and IV is better absorbed than another. The best absorption occurs when the drug is given on an empty stomach (Stewart and May, 1947). Much larger doses have to be given orally than by injection, and it is generally accepted that some five times the injected dose has to be given orally in order to produce the same effect, which even then is not quite as reliable. On the basis of their experimental work in man, Hoffman and Volini (1947) suggest an initial dose of 200,000 units followed by 100,000 units every three hours.

Oral administration has been used successfully in the treatment of pneumonia (Anderson and Landsman, 1947) and of gonorrhœa (Bushby and Harkness, 1946). These latter authors obtained as good results as are obtained with systemic administration, by giving six doses of 40,000 units over a period of 15 hours. They emphasize the importance of restricting the fluid intake during

the period of treatment. Tablets of penicillin have been very successfully used in the prophylaxis of gonorrhœa. With a single dose of 250,000 units the occurrence of the disease in men exposed to infection was almost completely prevented (Eagle *et al.*, 1949). Oral penicillin is, of course, of particular value in infants, as an

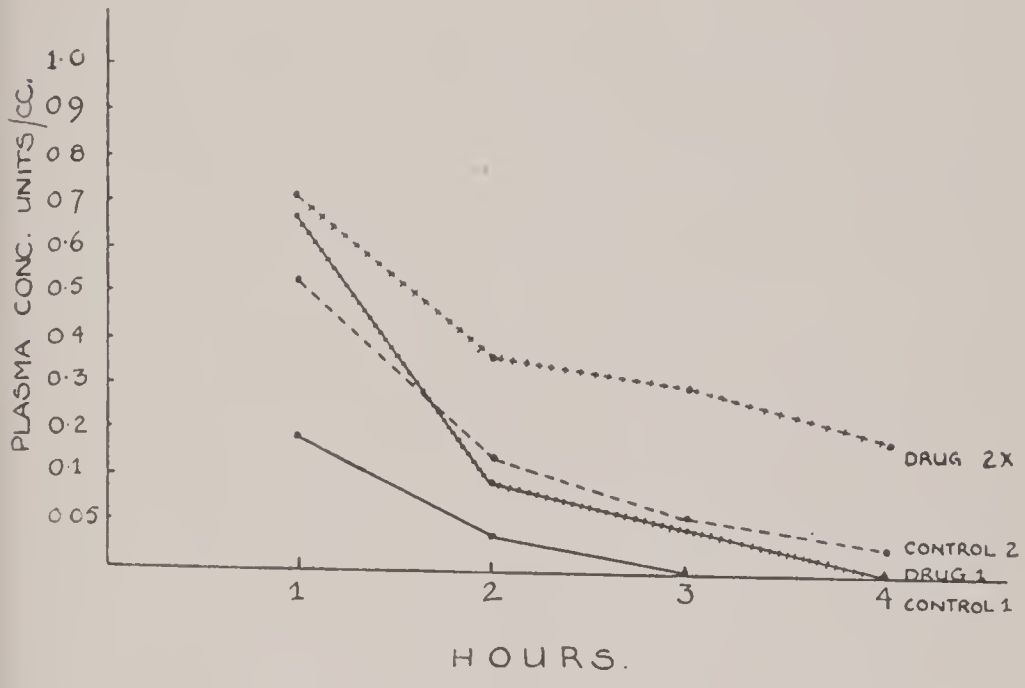


FIG. 15

Showing the effect of carinamide on the plasma level of penicillin given orally (100,000 units).

Control 1 and control 2 : Separate curves for penicillin alone.

Drug 1. 0.5 gram carinamide given orally 1 hour before penicillin administration.

Drug 2. 1 gram carinamide given orally 1 hour before penicillin administration.

(After Rapoport *et al.*, 1948.)

addition to the feeds, and in young children (Suchet-Kaye and Latter, 1947 ; Cohlan *et al.*, 1948). Simultaneous administration of oral penicillin with carinamide markedly increases the resulting blood concentrations as is shown in Fig. 15 (Rapoport *et al.*, 1948). This method of administration will no doubt be further investigated clinically.

Mode of Action

This is discussed by Pratt and Dufrenoy (1948). Penicillin can produce bacteriostatic, bactericidal or bacteriolytic actions but the mechanism by which these effects are produced has by no means been elucidated. It is generally accepted, however, that the mechanism is a different one from that by which sulphonamides produce their bacteriostatic action, which is discussed in Chap. 15. The action of penicillin, unlike that of the sulphonamides, is not greatly affected by the presence of pus or of organic matter and this is indeed one of the great advantages of the drug. It has been shown by Chain and Duthie (1945) that very small concentrations of penicillin markedly inhibit and ultimately stop the oxygen uptake of staphylococci during the stages of multiplication of the organisms, while no such effect is seen during the "resting phase." Penicillin thus appears to interfere with the metabolic functions of micro-organisms, at least during the early stages of development. Studies with the electron microscope show that, under the influence of penicillin, numerous diplococcal and tetrad forms can be seen immediately before the division of haemolytic streptococci (Holzer, 1947), showing that normal and full bacterial division does not occur. Preliminary investigations with penicillin labelled with radioactive sulphur suggested that organisms immersed in solutions of the drug did not absorb any appreciable amount of it (Rowley *et al.*, 1948), but later investigations have shown that penicillin is taken up and is indeed strongly and irreversibly attached to the organisms. There is also some evidence that sensitive organisms take up more of the drug than resistant strains (Cooper and Rowley, 1949). Pasynskii and Kastorskaya (1947) have shown that a single bacterial cell of staphylococcus aureus adsorbs 1,000 molecules of penicillin. This is of the same order as the amount of sulphonamide adsorbed by bacteria.

One of the earliest effects of penicillin on staphylococcus aureus appears to be the blocking of the absorption of an essential metabolite, glutamic acid (Gale, 1947; Gale and Taylor, 1947). This occurs only during the period of growth, and not in resting cells. The effect is particularly interesting, since glutamic acid is a component of glutathione, which plays such an important part in cell metabolism by virtue of its sulphydryl (-SH) group. Pratt and Dufrenoy (1948) believe that penicillin has an important action by promoting dehydrogenation of -SH groups to S–S, and produces

this effect more rapidly than the organism can restore the active sulphydryl group: the organism is thus put into a state of dehydrogenation which interferes with its metabolism and is ultimately lethal. According to this view, therefore, penicillin produces its effect by interfering with the transfer of hydrogen in the cell.

It is possible, however, that other enzyme systems may also be affected. It has been shown that the bacteriostatic action of penicillin is antagonized by nucleic acid (Pandalai and George, 1947) and it is possible that the drug produces its effect by interfering with phosphorylation mechanisms which are also fundamental to the life of the cell. This would explain why the supply of suitable phosphorylated compounds, such as nucleic acid, interferes with the action of penicillin.

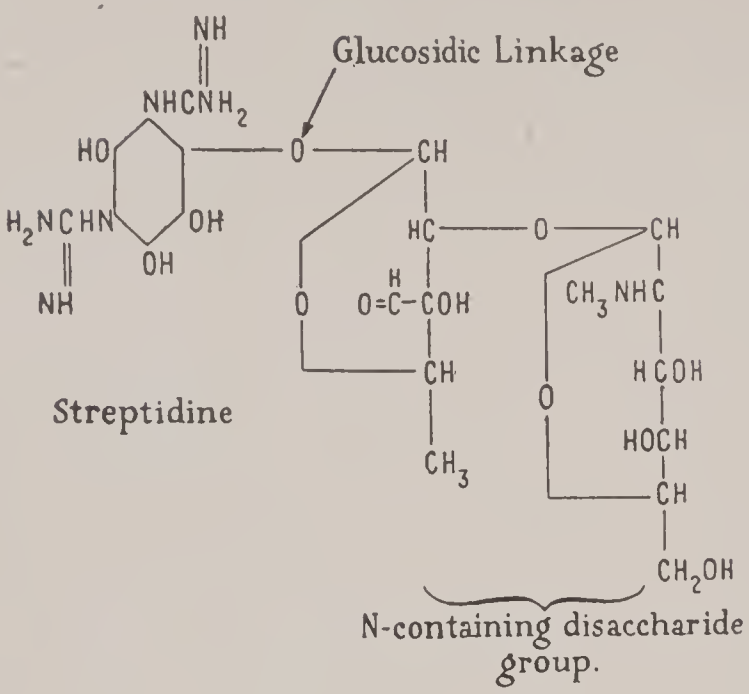
Other effects of penicillin which have been described are an inhibition of the dephosphorylation of nucleotides (Gros and Machebœuf, 1948) and an inhibition of the oxidation of nucleic acid (Krampitz and Werkman, 1947). The data of Mitchell (1949) are also consistent with the view that penicillin reacts initially with components of the bacterial cell involved in nucleotide dissimilation.

Streptomycin

This was discovered in 1944 in America by Waksman and his colleagues in a systematic search of soil micro-organisms for substances suitable for clinical application. It is produced in fluid cultures by an organism, *Streptomyces griseus*, which belongs to a group of Actinomyces having the general name, Streptomyces. It is interesting that Waksman actually isolated this organism from the soil as long ago as 1919. Streptomycin is effective against gram negative organisms but its main interest lies in the fact that it produces an action on *M. tuberculosis*.

Chemistry

Streptomycin is an organic nitrogenous base and its chemical constitution has been determined by Folkers and his co-workers (Kuehl *et al.*, 1947) in a remarkable piece of work. It consists of a hydroxylated base, streptidine, attached through a glycosidic linkage to a N-containing disaccharide-like group. The formula is shown on p. 150.



Streptomycin

It seems likely that there are several other substances closely related in chemical constitution to streptomycin, and one of these, called streptomycin B, has been isolated in a pure form by Fried and Titus (1947). Some of its biological properties have been investigated by Rake *et al.* (1947). They found that its toxicity is about the same as that of streptomycin, but that, weight for weight, it is about one third as active as streptomycin in experimental tubercuIous infections in mice. The nomenclature of streptomycin preparations is discussed by Waksman (1948). By the use of paper partition chromatography Winsten and Eigen (1948) have shown that there are at least five components in the streptomycin complex.

By the catalytic hydrogenation of streptomycin, probably at the carbonyl group, Peck *et al.* (1946) have made *dihydro-streptomycin* which has properties very similar to streptomycin but appears to be less toxic (see several papers in *Amer. Rev. Tuberc.* 1948, 58, 479–536). Both the sulphate and hydrochloride of dihydro-streptomycin have been obtained in crystalline form (Solomons and Regna, 1949; Wolf *et al.*, 1949). In a study by Hobson *et al.* (1948), vestibular damage appeared later and less

uniformly with 5 grams of dihydrostreptomycin daily than in patients treated with 3 grams of streptomycin daily. Another advantage of the new compound is that it is well tolerated by subjects in whom allergic reactions are produced by streptomycin. The hydrogenated compound is, however, ineffective against tubercle bacilli which have become resistant to the parent compound. The irritation produced by dihydrostreptomycin at the site of injection is appreciably more severe than that produced by streptomycin and it is not advisable to use the new drug intrathecally for the treatment of meningitis (Hinshaw *et al.*, 1948).

More recently still Waksman and Lechevalier (1949) have isolated a substance, called *neomycin*, from another strain of streptomyces (streptomyces fradiæ). Its activity is probably due to more than one chemical entity and it may thus be defined as a "neomycin-complex." One of the active substances, designated as neomycin A, has been isolated in a crystalline form as a sulphonic acid salt (Peck *et al.*, 1949). Neomycin is active against streptomycin resistant tubercle bacilli, and resistance of tubercle bacilli to neomycin develops less rapidly than to streptomycin (Waksman *et al.*, 1949). Neomycin may thus prove to be of great value in the treatment of tuberculosis.

The unit of streptomycin has been defined as the activity of 1 μg. of pure streptomycin base. There is also a working standard with an activity of 400 units per mg. Experimental lots have been prepared which have a potency of 900 units per mg. Streptomycin is freely soluble in water but not in organic solvents and is much more stable than penicillin. Solutions kept in the refrigerator deteriorate only slowly, and even at room temperature they may be kept for several days without loss of potency.

Absorption and Fate

Streptomycin is poorly absorbed from the intestine, though unlike penicillin it is not inactivated in the gut; it must thus be administered by injection, usually intramuscular. It can, however, also be applied locally. After injection it is distributed throughout the extracellular fluids and rapidly excreted, the rate of excretion varying in different species. Marshall (1948) investigated the fate of the drug in man, using a sensitive chemical method for the determinations, as described by Marshall *et al.* (1947). He found that after intramuscular injection of 20 mg. per kg., the

maximum blood concentration is reached in 1–2 hours and then falls rather rapidly (see Fig. 16).

According to the data of Boxer *et al.* (1948) the concentration of streptomycin produced in the blood is proportional to the dose per kilogram of body weight, within a definite range of dosage. From the results obtained, it is possible to calculate with reasonable accuracy the serum concentrations of the drug in man after intramuscular injection of streptomycin in doses ranging from 4,000 to 20,000 μg./kg. Much of the drug is excreted in the urine,

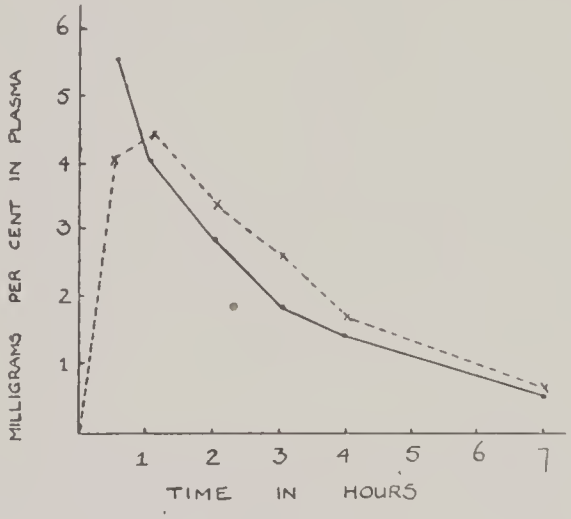


FIG. 16

Showing the plasma levels of streptomycin following its intravenous and intramuscular administration in man (20 mg./Kg.). (After Marshall, 1948.)

.———. Intravenous injection.
×- - - -× Intramuscular injection.

but this varies in different subjects and in some cases a considerable amount of the dose given is not accounted for by urinary excretion. In dogs most of the drug is excreted by the kidneys. Its excretion appears to be essentially by glomerular filtration. The drug is also concentrated and excreted by the liver into the bile, and can pass through the placental barrier into the fœtal circulation (Zintel *et al.*, 1945), though the concentration in the cord blood and amniotic fluid is generally less than half that found in the maternal blood (Woltz and Wiley, 1945).

When streptomycin is given intramuscularly very little diffuses into the cerebrospinal fluid unless very large doses are given. In acute meningitis, however, much greater diffusion occurs and effective chemotherapeutic concentrations can be produced with the usual therapeutic doses (Zintel *et al.*, 1945). Even then the concentration is less than half that found in the blood serum (Adcock and Hettig, 1946). The drug does not readily penetrate into the ocular fluids and very large doses have to be given to produce chemotherapeutic concentrations in the anterior chamber of the eye (Leopold and Nichols, 1946). Streptomycin penetrates into tissues when applied locally, but its penetration into the cornea is poor; this can, however, be improved by the use of a wetting agent (Bellows and Farmer, 1947).

Streptomycin can safely be given intrathecally and is then retained in the cerebrospinal fluid for periods up to 30 hours. During the first few hours very high concentrations are present, and, even after 24 hours, 20 or more units per ml. have been found in the cerebrospinal fluid following the injection of 20 mg. of the drug (Kolmer, 1947; Medical Research Council, 1948).

Toxicity

Streptomycin is a substance with a definite toxicity which, as will be seen later, limits the doses, even of pure material, that can be used clinically. The toxicity and actions in animals of streptomycin preparations of various degrees of purity have been investigated by Molitor *et al.* (1946). They found great variations in the toxicity of different preparations. Pure streptomycin had a L.D.50 in mice of about 1.2 G./kg. when given subcutaneously. Animals showed respiratory distress and ultimately died of respiratory failure. The effects produced by chronic administration vary in different species. For example, monkeys exhibit some impairment of renal and hepatic function while dogs show, in addition, cerebellar and labyrinthine disturbances.

Impure preparations produce a histamine-like fall in blood pressure in cats, and a temporary antidiuretic effect in rats. These effects are not seen with pure preparations.

Activity *In Vitro*

Streptomycin is effective against certain acid fast bacilli notably M. tuberculosis, and against gram negative and gram

positive organisms. It is usually less active than penicillin against gram positive organisms, so that its main use is in certain types of tuberculosis and in infections with some gram negative organisms.

The gram negative organisms which are susceptible include B. coli, S. typhi, Br. tularensis, B. friedländeri, Brucella abortus, H. influenzæ, Proteus vulgaris, S. paratyphi B., N. gonorrhœa, H. pertussis, Past. pestis and Ps. æruginosa; and the gram positive organisms include staphylococci, hæmolytic streptococci, actinomycetes and some spore forming ærobes. The anærobes, such as Cl. tetani and Cl. Welchii, are highly resistant to the drug. The susceptibilities of a number of organisms are given in Table 3. The chemotherapeutic concentration that can be maintained in the body fluids of man is of the order of 5 μg. per ml.

The activity of streptomycin is decreased in an acid medium and the drug may therefore be less effective in conditions where there is tissue damage which may produce an acid reaction (Abraham and Duthie, 1946).

Activity in Experimental Infections

The most extensive and important experiments have been carried out in animals infected with tuberculosis. The therapeutic value of the drug can be clearly demonstrated in infected guinea pigs. If the treatment is started even several weeks after inoculation it will greatly lengthen the life of the animals. On post mortem examination such animals may show no macroscopic or even microscopic signs of infection; lesions may be present but they are usually inactive and characterized by fibrosis and calcification (Feldman *et al.*, 1945). An effect can be demonstrated within three weeks of the beginning of treatment. The value of streptomycin can also be demonstrated in animals (mice and guinea pigs) inoculated intravenously, even with large doses of organisms. Feldman and Hinshaw (1948) emphasize the formidable character of such a widely disseminated infection, and suggest that in the future new substances should be tested in animals in which tuberculous infection has been induced by intravenous inoculation. Such a procedure should provide valuable data for comparison with the effectiveness of streptomycin. Methods for evaluating the activity of antituberculous drugs in animals are reviewed by Raleigh and Youmans (1948).

In spite of these very striking actions, however, streptomycin does not usually kill off all the organisms in the treated animals. Even after many months of treatment and in the absence of any signs of infection, the presence of fully virulent tubercle bacilli can be demonstrated in the guinea pigs by suitable methods (Feldman and Hinshaw, 1948). This has also been clearly demonstrated in experimental corneal infections in the rabbit : by treatment with streptomycin such lesions will heal, but, on section of the cornea, tubercle bacilli can still be found in the lesion (Gardiner *et al.*,

TABLE 3

Anti-bacterial Activity of Streptomycin.

Gram-positive		Gram-negative	
Organisms	Microgram per ml.*	Organisms	Microgram per ml.*
A. bovis	3.75	Bact. aerogenes	0.5 – 2.5
B. anthracis.	0.375	B. mallei	10.0 –>10.0
B. megatherium	0.25 – 3.0	Br. abortus	0.5 – 3.75
B. mesentericus	1.67	Br. melitensis	0.5
B. mycoides	0.1 – 3.8	Br. suis	0.5
B. subtilis	0.12 – 1.0	S. typhi	1.0 – 37.5
Cl. butyricum	8.34	B. coli communis	0.3 – 3.75
Cl. welchii	>104.0	B. coli communior	1.0 – 4.0
Cl. septicum	>105.0	H. influenzæ	1.56 – 5.0
Cl. sordelli	>105.0	H. pertussis	1.25 – 3.0
Cl. tetani	>104.0	B. friedländeri	0.625– 8.0
C. diphtheriæ	0.375– 3.75	B. ozænæ	0.375– 1.5
Str. pneumoniæ	8.0	N. gonorrhœæ	5.0
Myco. tuberculosis (hominis)	0.15	N. meningitidis.	5.0
Staph. aureus	0.5– >16.0	Past. lepiseptica	0.5 – 2.5
Str. fæcalis	50.0	Past pestis	0.75 – 1.5
Str. hæmolyticus	2.0– >16.0	Br. tularensis	0.15 – 0.3
Str. lactis	4.0	Proteus vulgaris	0.4 – 3.0
Str. salivarius	5.0 – 25.0	Ps. aeruginosa	2.5 – 25.0
Str. viridans	>16.0	S. typhi-murium	4.0 – 10.0
		S. enteritidis	0.5
		S. paratyphi B.	2.0
		Chr. prodigiosum	1.0
		Shig. flexneri	0.25 – 3.75
		V. choleræ	6.0 – 37.5

* Approximately smallest amounts of streptomycin per ml. of suitable culture media for bacteriostatic activity ; one microgram (0.001 mg.) equivalent to one unit. (From Waksman and Schatz, 1945.)

1949). The effectiveness of streptomycin may be enhanced by combining with it a drug, such as potassium iodide, which makes the bacteria more accessible to the chemotherapeutic agent (Woody and Avery, 1948).

The value of streptomycin in certain other infections, and notably those with gram negative organisms, can also be shown experimentally. For example, Heilman (1944) found that mice inoculated with Past. tularensis and treated with streptomycin for ten days all survived, while all the control animals died. Robinson *et al.* (1944) were able to save a large percentage of mice inoculated with a lethal strain of S. schottmulleri by a single intravenous or subcutaneous dose of the drug given immediately after the inoculation. The striking value of the drug in experimental pneumonic and bubonic plague in mice has also been demonstrated (Quan *et al.*, 1947).

Clinical Applications

The main use of streptomycin has been in the treatment of certain forms of tuberculosis (see Official Memorandum, 1949), and of some infections with gram negative organisms, i.e. mainly those conditions in which penicillin or sulphonamides are of little or no value. Unfortunately the drug is by no means always effective: this is frequently due to the presence in the lesions of naturally resistant strains of a particular organism, or to the rapid development of resistance by the organisms during the course of the treatment.

Dosage and Methods of Administration

Unlike penicillin, streptomycin can produce serious toxic effects and hence the dose should be restricted to the minimum which will be therapeutically effective. It has, indeed, been strikingly shown that an increase in the daily dose beyond a certain level markedly raises the incidence of toxic effects (Madigan *et al.*, 1947). The drug is not well absorbed when given orally and the intramuscular route is usually the method of choice. As a rule, doses of the order of 0.5 grams every six hours are sufficient, since the drug is excreted much more slowly than penicillin (Loewe and Alture-Werber, 1947). Larger doses (3 grams per day) of a highly purified preparation have, however, been given over long periods (Farrington *et al.*, 1947) without undue toxic effects.

and it seems likely that such higher doses will be used, when necessary, as the purer preparations become available. In the treatment of pulmonary tuberculosis, doses of 1 gram per day have frequently been used.

The drug can also be given intrathecally in doses up to 0.1 gram per day. Larger amounts may be well tolerated but can produce severe toxic effects and should therefore not be given. (Farrington *et al.*, 1947). Local injections have also been given for infections in the thorax and in the abdomen.

Topical applications of streptomycin, in solutions or in ointment, may be used in the treatment of wounds and burns, and solutions may be used for irrigating cavities such as the bladder in cases of cystitis. Solutions or ointments can also be used for the treatment of infections of the conjunctival sac and in the anterior part of the eye. Streptomycin can safely be applied in concentrations of 10 mg. per ml., even in the conjunctival sac (Bellows and Farmer, 1947).

Toxic Effects

These have become less as purer preparations have become available. Farrington *et al.* (1947) have used material of at least 95 per cent purity over long periods, probably the purest streptomycin so far employed in a thorough clinical trial, and reference to their results will therefore be made frequently.

Four types of toxic reactions have been observed with streptomycin namely, (1) A histamine-like effect, characterized by flushing, headache and an abrupt fall in blood pressure. (2) Various allergic reactions, which are not usually serious. Farrington *et al.* found it necessary to interrupt treatment in only two out of 31 cases because of the development of allergic reactions, and in both of these it was eventually found possible to resume the treatment. The reactions consisted of an itching maculopapular eruption over the trunk and extremities ; a rise in temperature and nausea and vomiting ; and leucopenia. These investigators also observed that many of their cases developed an eosinophilia, but the significance of this effect is not yet clear. (3) Neurological disturbances involving vestibular dysfunction and occasionally producing deafness. (4) Renal irritation manifested by cylindruria and occasionally accompanied by impairment of renal function.

With the exception of the histamine-like reactions, all these effects have been obtained with pure preparations of the drug and

are in all probability actually due to streptomycin itself (Farrington *et al.*, 1947). The histamine-like reactions are probably due to impurities. Streptomycin may produce irritation at the site of injection, but this is considerably less with purified preparations than with the crude drug.

The symptoms of vestibular dysfunction, e.g. vertigo, appear on about the 21st day after the beginning of treatment, but complete recovery occurred in both cases observed by Farrington *et al.* This is probably due to compensatory mechanisms rather than to the restoration of labyrinthine function. Recovery is, however, not always complete and it has been suggested that this may be especially so in old people (Brown and Hinshaw, 1946). All cases of deafness in the series treated by Farrington *et al.* developed in patients in whom the drug was given intrathecally or in the presence of well defined renal insufficiency, and these authors suggest that this reaction may be the result of over-dosage. This conclusion is also supported by the findings of Brown and Hinshaw (1946) who found that deafness developed only in cases where the drug was given intrathecally, or where the daily intramuscular dose was 5 grams or more. Fowler and Seligman (1947) have made special examinations of auditory and vestibular functions in patients treated with streptomycin, and find that slight auditory disturbances occur quite frequently. Streptomycin is, of course, frequently used in the treatment of meningitis which itself may be responsible for the deafness which develops in some cases.

Renal toxic effects were seen quite frequently by Farrington *et al.* In most cases the excretion of casts occurred within 48 hours of the beginning of therapy, but occasionally it did not occur till later. This effect was much more marked when the urine was acid, and was slight or absent when the urine was maintained neutral, or slightly alkaline, by the administration of sodium bicarbonate. Renal function was only permanently affected in one out of 31 patients. Dihydrostreptomycin appears to be less toxic than streptomycin to the vestibular apparatus and can replace the latter drug in patients hypersensitive to it (*Amer. Rev. Tubere.*, 1948, **58**, 479–536). It may become the drug of choice in cases in which prolonged treatment is necessary.*

* Later results makes this rather doubtful (see Minutes, Seventh Streptomycin Conference, Veterans Administration, 1949).

Meningeal and Miliary Tuberculosis

Before the advent of streptomycin, *tuberculous meningitis* was invariably fatal and hence the effects of the drug have been particularly dramatic in this condition, though only a minority of patients treated make a satisfactory recovery. Several investigations have now been performed and certain facts are becoming clear (Medical Research Council, 1948; Mollaret, 1948). Early diagnosis is of great importance and the ultimate results are much better when the treatment can be started in the early stages. The combined administration of the drug intrathecally and intramuscularly gives better results than are obtained by giving it by the intramuscular route alone. And the results are definitely worse in children under three years of age than in the older children. The patient may respond well and yet relapse, sometimes many months later. A further point of importance is that when complete spinal block develops at an early stage of treatment, streptomycin should be administered by the cisternal or ventricular route instead of by the lumbar route.

TABLE 4

Results of Streptomycin Therapy of Tuberculous Meningitis

No. of cases admitted before August 18th, 1947	Condition on March 15, 1948			
	Good	Stationary or relapsed	Deteriorating	Dead
105	27 (26%)	6 (6%)	1 (1%)	71 (67%)

(From Medical Research Council, 1948)

There has been a good deal of discussion as to how long the treatment should be continued. The present tendency is to treat for some three months, but this does not prevent a number of cases from relapsing, and some investigators have given the drug for many more months; others believe a shorter period of treatment will give equally satisfactory results. This question awaits further investigation of the mode of action of streptomycin, and especially of the development of resistance by the organisms (discussed on pp. 164–166).

In *miliary tuberculosis* the results are better and a majority of the patients treated with streptomycin show a complete radiological clearing of the pulmonary lesions (Council on Pharmacy and Chemistry, 1947).

Other Forms of Tuberculosis

Favourable results have also been obtained with streptomycin in other forms of tuberculosis. It seems clearly of value in healing ulcerative or granulomatous lesions of the tracheobronchial tree or larynx; it is strikingly effective in tuberculous sinuses, even when these are multiple and originate in lesions of bone, cartilage or lymph nodes; it appears to be valuable in the treatment of tuberculosis of the alimentary tract and of tuberculous peritonitis, and certainly affords relief in cases where these lesions are associated with continuing pulmonary infection (Mason *et al.*, 1949). The oral administration of the drug in such cases deserves further investigation (Council on Pharmacy and Chemistry, 1947). Beneficial effects have also been demonstrated in certain forms of *pulmonary* tuberculosis. The exudative phase of the infection responds most readily, while fibrotic lesions remain unchanged. In properly selected cases streptomycin, in conjunction with collapse therapy, enhances the prospects of clinical arrest of the disease. It also affords protection against spread of infection post-operatively following thoracoplasty (Streptomycin Committee, 1947; Pulaski and Seeley, 1948; Council on Pharmacy and Chemistry, 1948). A daily dosage of 1 gram, given as two intramuscular injections of 0.5 gram at 12 hourly intervals, is considered to be sufficient in pulmonary tuberculosis. Higher doses, viz. 2 grams per day, have been used in trials of the drug in patients with acute progressive bilateral pulmonary tuberculosis, unsuitable for collapse therapy (Medical Research Council, 1948). This was a carefully controlled experiment and the results show that the treatment was of benefit, especially in patients clinically acutely ill on admission. Improvement was greatest in the first three months after which many of the cases began to deteriorate. This was probably due to the development of streptomycin resistance which was clearly demonstrated in a number of cases. One year after the beginning of treatment there was still clear evidence of the value of the treatment, as shown by the figures given in Table 5. The difference in mortality between the two

groups is statistically significant (see Chap. 17). The development of resistance in relation to response to treatment is discussed by Crofton and Mitchison (1948). A remarkable subsequent finding is that there exists a striking correlation between the incidence of resistant tubercle bacilli and the presence of caseation and cavitation in patients treated with streptomycin. For example Howlett *et al.* (1949) found strains uninhibited by 10 μg. of streptomycin per ml. of culture medium in 33 (59 per cent) out of 56 patients with frank caseation or cavitation, and no such resistant strains in 31 patients who had no frankly caseous or cavernous lesions prior to treatment. Similar results have been obtained by Howard *et al.* (1949).

TABLE 5

Condition of patients 12 months after beginning of treatment as compared with condition on admission.

Group	Total	Improvement	No change	Deterioration	Death
Streptomycin treated	55 100%	31 56%	4 7%	8 15%	12 22%
Control	52 100%	16 31%	5 10%	7 13%	24 46%

A recent preliminary report on a clinical trial by the Medical Research Council (1949) records a very important finding. The combination of P.A.S. (20 grams per day) with streptomycin (1 gram per day) in the treatment of acute forms of pulmonary tuberculosis reduces considerably the risk of development of streptomycin-resistant strains of tubercle bacilli during the six months following the start of treatment. It is not yet known whether the same results are obtainable in other forms of tuberculosis amenable to streptomycin therapy. These high doses of P.A.S. are not well tolerated by some patients and it remains to be seen whether the same effect can be produced with lower doses of P.A.S. It is possible that the chemical compound of P.A.S. and streptomycin described by Hobby *et al.* (1949) may prove even more satisfactory.

Favourable results have also been obtained with streptomycin

in more chronic forms of indolent pulmonary tuberculosis of limited extent and severity, which had not been arrested by long periods of treatment by conventional means (Howlett and O'Connor, 1948). The drug has also been used in tuberculous bronchopneumonia in children (McLaren Todd, 1949).

The use of streptomycin has greatly decreased the risks of pulmonary resection for tuberculosis, lowering the mortality from 25 to 4.5 per cent and the occurrence of post-operative empyema from 12 to 4 per cent. The results in thoracoplasty are much less striking and it is suggested that streptomycin should be reserved for the occasional borderline cases in whom the disease is more widespread and more exudative in character than is usually considered suitable for surgery (Murphy, 1948).

Tanner and his co-workers (1948) have treated tuberculous cavities by local injection of streptomycin solution. The site of injection is carefully controlled by X-rays. The dose is 0.5 gram in 1 ml. daily. Higher concentrations are irritating and may even produce hæmorrhage. This treatment is combined with systemic administration of streptomycin, and should be used only in carefully selected cases. Its chief value is to prepare the patient for subsequent surgery.

Good results have also been reported with streptomycin (1 gram per day) in the treatment of tuberculosis of bones and joints (Bickel *et al.*, 1948). The results in tuberculosis of the genito-urinary system have not, so far, been very encouraging, though the drug will produce temporary improvement in the general status of most patients and will, at least for a time, clear the urine of viable tubercle bacilli (Abernethy and Edelbrock, 1949). In combination with calciferol, streptomycin has been very effective in the treatment of lupus vulgaris (Cornbleet, 1948).

Other Conditions

These are essentially infections of almost all kinds due to many species of *gram-negative bacilli*. Streptomycin is highly effective in the treatment of tularæmia, a disease due to the gram negative organism, P. tularense. In the acutely ill patient the effect is most dramatic (Berson and Harwell, 1948). The drug is also frequently successful in controlling the following other infections: (1) *Meningitis*, due to H. influenzae, B. coli, Ps. pyocyanea, Proteus, and Staphylococcus pyogenes. (2) *Septicæmia*, due to

B. coli and Ps. pyocyanea. (3) *Gonorrhœa.* (4) *Urinary tract infection* due to B. coli, Proteus, Ps. pyocyanea, Str. fæcalis and Staphylococcus pyogenes. (5) *Local sepsis* in which the following organisms are involved : B. coli, Proteus, Ps. pyocyanea, Staphylococcus pyogenes and Hæmolytic streptococcus (Wilson, 1948). In local sepsis the intramuscular administration of the drug may be combined with daily application of a saline solution (2 mg. per ml.). Experimental work on rabbits suggests that streptomycin might be of value in the treatment of acute intestinal obstruction by preventing the growth of bacteria in the devascularized bowel wall (Davis *et al.*, 1948).

It must be emphasized that in almost every type of infection the micro-organisms may rapidly become resistant. For example, only about half of the cases of urinary tract infections are controlled by streptomycin, and in these the beneficial effect is seen very rapidly. Failure is almost always due to the development of resistance and the treatment should usually not be continued for more than a few days, and second courses are useless. In general, courses of treatment should be planned to exert the greatest possible effect from the beginning and some 3 grams per day is usually given in adults. Owing to the short periods of administration, toxic effects are unusual and not serious. In the treatment of long standing urinary infections there are now two drugs which may be better than the sulphonamides—they are streptomycin and *mandelamine* (methenamine mandelate) (*British Medical Journal*, 1948). It remains to be seen whether streptomycin or mandelamine is the more satisfactory. According to a recent report by Duca and Scudi (1947) mandelamine does not readily give rise to resistant strains, and this also applies to organisms recently isolated from the urinary tract, which rapidly developed resistance to streptomycin and sulphathiazole (Scudi and Duca, 1949). The absorption, distribution and renal excretion of mandelamine have been studied by Scudi and Reinhard (1948).

Streptomycin also appears to be rapidly effective in the treatment of *plague* (Karamchandani and Rao, 1949), and encouraging results have also been obtained in some cases of *actinomycosis* in some of which other methods of treatment had failed (Torrens and Wood, 1949 ; Pemberton and Hunter, 1949). There is evidence that it may be of value in *infantile gastro-enteritis* when given orally in doses up to 0.1 gram per pound per day,

incorporated in milk (Pulaski and Seeley, 1948; James *et al.*, 1948), though the results of Holzel *et al.* (1949) have not been encouraging.

Mode of Action

It seems likely that streptomycin has both *bacteriostatic* and *bactericidal* actions (Smith and Waksman, 1947). High concentrations are markedly bactericidal and when these are attained they kill off organisms very rapidly (Garrod, 1948). This effect is of importance when the drug acts locally, as in the treatment of urinary infections or of infected wounds. It is usually accepted that a chemotherapeutic substance acts only when its concentration in the body fluids is above a certain level, and that no effect is produced when no demonstrable amounts of the substances are present. Corper and Cohn (1948) have, however, obtained evidence which suggests that, in the case of streptomycin, the effect persists after the blood concentration falls below the concentration to which the organism is sensitive. Thus they found in one experiment, that guinea pigs treated for some ninety days with 25,000 units of streptomycin daily and then injected intravenously with 1 mg. of virulent human tubercle bacilli one day after discontinuance of treatment, when streptomycin is not determinable in the blood, showed an increase of 29 days in average duration of life as compared with the untreated controls, whose average survival time was 22 days. This question obviously requires further investigation.

The mechanism by which streptomycin produces its effect is unknown, but research on this subject is actively proceeding and there are a number of interesting observations. Geiger (1947) found that the oxidation of amino acids by E. coli is greatly facilitated by permitting the cells first to act on fumarate or other carbon compounds, and that the increased ability of such E. coli cells to oxidize amino acids is prevented by streptomycin. According to Green *et al.* (1948) the action of streptomycin on some organisms, including E. coli, is antagonized by pyruvic and fumaric acid.

Geiger states that streptomycin does not influence the oxygen uptake of aerobic organisms in the presence of glucose but Berkman *et al.* (1948) have found that the drug interferes with the carbohydrate metabolism of resting bacteria. Henry *et al.* (quoted by

Geiger) reported at a Conference on Antibiotic Research that streptomycin has no effect on a number of enzymes of staphylococcus aureus, including cytochrome-oxidase, catalase, ærobic oxidations and anærobic fermentations of a number of carbon compounds, dehydrogenase systems, carboxylase, urease, and carbonic anhydrase, but produces a partial inhibition of the ærobic oxidation of glycerol. Bernheim and Fitzgerald (1947) observed that streptomycin prevents the oxidation of benzoic acid by M. tuberculosis.

There is also evidence that the effect of the drug is concerned with the metabolism of nucleic acid. Gros and Machebœuf (1948) have found that this is inhibited by streptomycin at two stages, viz., during the depolymerization of nucleic acids and during the liberation of mononucleosides. It has also been found that streptomycin, like the acridines, forms electro-absorption complexes with yeast nucleoprotein (Massart *et al.*, 1947; Gros and Rybek, 1948).

Development of Resistance

A feature of the action of streptomycin is that the organisms tend to become resistant to the drug. This is, of course, also seen with other chemotherapeutic substances like the sulphonamides and penicillin, but the effect is especially marked with streptomycin, and may indeed come on very rapidly, as is frequently seen in the treatment of urinary infections due to gram negative organisms, and can also easily be demonstrated *in vitro* by repeated subcultures of organisms in increasing concentrations of the antibiotic (Miller and Bohnhoff, 1946). The resistance so acquired by these organisms is permanent and there is indeed a danger that such strains, derived from a case in which treatment failed, may with increasing frequency be found in previously untreated patients. In tuberculosis too, the organisms tend to become resistant but here the process is slower, and the drug resistant strains cannot usually be demonstrated in appreciable numbers until streptomycin has been given for some 60–90 days (Feldman and Hinshaw, 1948; Streptomycin Committee, 1947). This would suggest that treatment beyond that period will usually not be of much value, though this assumes that the bacilli in the body are as resistant to streptomycin as they are *in vitro*, an assumption which has not been fully proved.

The mechanism by which bacteria become resistant to strepto-

mycin and other chemotherapeutic substances is unknown. It may be that a mutation occurs, or alternatively that the more susceptible cells are eliminated, leaving cells which possess a natural resistance. This second possibility may well explain the development of resistance to streptomycin, since this can occur very rapidly. Berkman *et al.* (1948) have shown that the carbohydrate metabolism of resistant bacteria is essentially unaffected by streptomycin whereas, as noted above, that of susceptible strains is inhibited by the drug. Both susceptible and resistant strains, however, absorb streptomycin equally at the cell surface, so that the change which occurs during the development of resistance is presumably not due to alterations in the surface properties of the organisms.

Another remarkable observation has been made which may throw some light on the mode of action of streptomycin and on the development of resistance. If organisms are grown in streptomycin, there develop not only variants which are resistant to the action of the drug, but others which will not grow at all in the absence of the drug, either *in vitro* or *in vivo*. Indeed, if such "dependent" strains of a meningococcus are inoculated in mice, they will not produce any lethal effect unless streptomycin is also administered in adequate doses, when the mice die of a fatal meningococcal sepsis. Meningococci recovered from the heart's blood of such animals grow readily on cultures made on streptomycin containing media, but not in duplicate cultures made on streptomycin-free media (Miller and Bohnhoff, 1947 ; Rake, 1948). It is possible, as has been suggested by Paine and Finland (1948), that in sensitive strains streptomycin interferes with some essential metabolite, presumably related in chemical structure, whereas dependent strains, on the other hand, utilize streptomycin as an essential metabolite or growth factor.

Other Drugs Used in Tuberculosis

Apart from streptomycin, certain other substances have been tried experimentally and clinically in the treatment of tuberculosis ; they are various *sulphones*, *p-aminosalicylic* acid and *semicarbazones*. Combinations of these drugs have also been investigated. The results with sulphones have, on the whole, not been encouraging but p-aminosalicylic acid, although still in the experimental stage, appears to deserve further trials.

Buu-Hoï *et al.* (1949) have made derivatives of phthalylhydrazine and claim that some of these, e.g. the isoamyloxy-1-phenyl-3 phthalazone-4, are more active than streptomycin in mice inoculated with a human strain of M. tuberculosis. Lupulon, a crystalline fat soluble substance obtained from hops, has also shown appreciable activity in experimental tuberculosis in mice (Chin *et al.*, 1949).

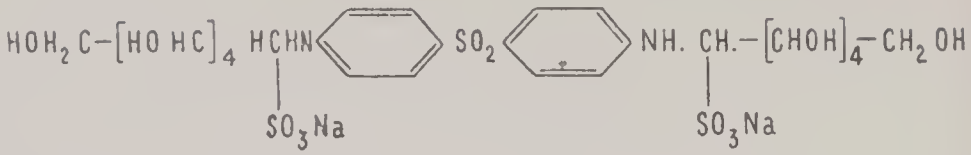
Sulphones

The chemotherapeutic activity of *diaminodiphenylsulphone* was discovered quite early in the investigations on sulphonamides by Buttle and his co-workers (1937), who demonstrated its value in streptococcal and pneumococcal infections in mice. Diaminodiphenylsulphone is, however, too toxic for clinical use,* and various derivatives have been made in order to decrease its toxicity and, if possible, enhance its activity. Following the report of Rich and Follis (1938) that sulphanilamide had some effect on experimental tuberculous infections in guinea pigs, Feldman and his co-workers (1942) tested the effect of the didextrose sulphonate derivative of diaminodiphenylsulphone (promin) and found that it prolonged the life of guinea pigs infected with tuberculosis. Other sulphones which have given interesting results are diasone, the disodium formaldehyde sulphoxylate derivative (Feldman *et al.*, 1943); promizole (diaminophenyl-thiazole sulphone) which embodies a thiazole grouping replacing one benzene nucleus in a diaminodiphenylsulphate type of structure (Feldman *et al.*, 1944); and sulphetrone, a tetrasulphonate derivative of diaminodiphenylsulphone (Brownlee, 1948). The formulæ of some of these compounds are given below :—

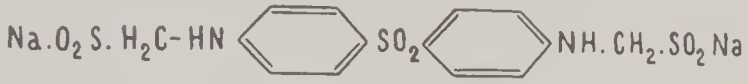
Formulæ of Sulphones

Diaminodiphenylsulphone

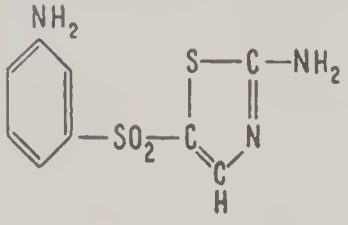
* Lowe, A. (1950, *Lancet*, i, 145), has found that, with suitable doses, diaminodiphenyl sulphone can safely be given orally without producing toxic effects of any consequence, and has successfully used it in the treatment of leprosy.



Promin



Diasone



Promizole

Other sulphones have been investigated by Youmans and Doub (1946) and by Hoggarth and Martin (1948). It has been clearly established that these compounds retard the progress of experimental tuberculous infections, but they are not capable of eliminating the organisms from animal tissues.

Eiseman (1948) has shown that by incorporating sulphones at one end of a surface active molecule, a marked increase in the *in vitro* effect on the tubercle bacillus can be obtained. He suggests that this is due to a concentration of the drug molecules at or beneath the mycobacterial cell boundary and that this increase in tuberculostatic activity might also be achieved with streptomycin. It still remains to be seen whether this enhancement of activity is obtained when the drug is used *in vivo*.

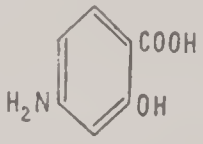
The use of the sulphones in the treatment of tuberculosis in man has not given any very convincing results. For example, Madigan (1948) found that sulphetrone produced no beneficial effects in acute infections, though some improvement was obtained in chronic lesions. Other clinical investigations are reported by Hinshaw *et al.* (1943) and by Petter and Prenzlau (1944). Further trials may indicate more clearly in which types of cases these drugs can be of value.

There is some evidence that sulphones and streptomycin produce a synergistic action on tuberculosis in guinea pigs

(Callomon *et al.*, 1946 ; Smith *et al.*, 1947 ; Brownlee and Kennedy 1948) and this seems quite possible in view of the different modes of action of these substances. Such a combination has also been used clinically by Madigan *et al.* (1946) and by Lincoln and Kirmse (1949) ; the latter authors conclude that the addition of promizole to streptomycin is of value in the treatment of miliary tuberculosis and tuberculous meningitis.

The sulphones have also been used in the treatment of *leprosy* and the results in this condition seem definitely encouraging (*Lancet*. 1948). Faget and Erickson (1948) indeed state that, at present, these drugs must be considered the optimal treatment of leprosy. Cochrane *et al.* (1949), in a review of their experience with various sulphones, state that a 50 per cent solution of sulphetrone in water is the best preparation at present available ; the dosage they suggest is 7 ml. subcutaneously twice a week. (See also footnote on p. 167.)

P-aminosalicylic Acid (P.A.S.)



p-aminosalicylic acid

The discovery of this substance was made as a direct result of fundamental work on the metabolism of tubercle bacilli. Bernheim (1941) found that benzoic acid and salicylic acid increased the oxygen uptake of these organisms in a more or less specific manner, thus suggesting the possibility that they, or similar substances, might be important metabolites of the tubercle bacilli. This led Lehmann to study the antitubercular action of a large series of structurally related compounds in the hope of finding some which would inhibit the bacterial metabolite by a mechanism of substrate competition. He found that of all the compounds studied, p-aminosalicylic acid (P.A.S.) was the most active *in vitro* against a bovine strain of tubercle bacilli (Lehmann, 1946). These results have been substantially confirmed : Youmans *et al.* (1947), for example, found that the drug is highly bacteriostatic *in vitro* for virulent human tubercle bacilli and that its activity is partly reversed by p-aminobenzoic acid and, according to Iványovics (1949), by high concentrations of salicylic acid. Lutz (1949) has found that the action of P.A.S. is also reversed by pantothenic acid and hence does not regard the reversal by

p-aminobenzoic acid as specific in nature. Goodacre *et al.* (1948) have investigated a number of derivatives and analogues of P.A.S.; no marked increase in activity was found. P.A.S. and streptomycin appear to act synergistically on tubercle bacilli *in vitro* (Vennesland *et al.*, 1948), and this has also been found in experimental tuberculous infections in mice (Moeschlin *et al.*, 1948). Additive effects between the two drugs have also been described in experimental infections of the rabbit's cornea and mouse's cornea (Rees and Robson, 1949, 1950) and of the guinea pig (Bloch *et al.*, 1949). Some data on the combined value of the drugs used clinically in miliary tuberculosis are given by Cartensen and Söderhjelm (1948).

Experimental Infections

The value of P.A.S. has also been demonstrated in infected animals, although the results appear to be greatly dependent on the size of the inoculum. Feldman *et al.* (1947) found that it exerted a favourable influence on the course of tuberculous infections in guinea pigs while McClosky *et al.* (1948), using a larger inoculum, failed to detect any appreciable therapeutic action in guinea pigs and rabbits. It has been clearly demonstrated that P.A.S. is of value in experimental tuberculosis in mice (Youmans *et al.*, 1947), though not as effective as streptomycin (Moeschlin *et al.*, 1948; Levaditi *et al.*, 1948).

Absorption and fate in the body. P.A.S. has the great advantages that it can be given orally, being well absorbed in the intestine, and that it has a low toxicity and can thus safely be given in large doses (McClosky *et al.*, 1948). Methods for its estimation in blood and body fluids have been described by Marshall (1948) and Dickenson and Kelly (1949). Its metabolism in various species has been studied by Way *et al.* (1948) and by Venkataraman *et al.* (1948). After an oral dose of 4 grams in man a maximum plasma level of the order of 7.5 mg. per cent is reached in 1–2 hours and this falls to less than 1 mg. per cent within 6 hours. About 85 per cent of the dose can be accounted for in the urine within 10 hours; in man some 60 per cent of this material is recovered in the urine in the form of conjugated amines. Ragaz (1948) has demonstrated that the conjugation of P.A.S. is increased when acid is administered and suggests therefore that the drug should be given therapeutically as the sodium salt and not as the free acid. The mechanism of

renal exeretion was investigated and it was found that about 25 per eent of the drug is filtered by the glomeruli and the remainder seereted by the tubules. This tubular exeretion can be halved by the administration of carinamide, and the therapeutic applieation of this finding is being investigated by Horne and Wilson (1949).

Alin and Helander (1948) have investigated the distribution of P.A.S. in tissues, taking advantage of the faet that it gives a strong green fluoreseenee in ultra violet light. They showed that the substance is aeeumulated in elastie fibres. In and around fresh lung tubereles in miee there was less P.A.S. than in the rest of the pulmonary tissue, partly due, they believe, to the destruetion of the elastic conneetive tissue.

Clinical Use. There are a number of clinical reports on the use of P.A.S.: *acute miliary* and *meningeal tuberculosis* are not affected but there is evidenee that it is of value in the exudative forms of *pulmonary tuberculosis* (Erdei, 1948; Nagley and Logg 1949; Carstensen and Sjölin, 1948); also, given by loeal instillation in eoncentrations up to 10 per cent, in tuberculous empyemata and in tuberculous fistulæ (Steinlin and Wilhelmi, 1948). Aeeording to Nagley and Logg (1949) treatment with P.A.S. often has the important effeet of making patients fit for eollapse therapy. The drug is given orally in doses of the order of 12-20 G. per day. As a rule it produees few toxie effeets; gastro-intestinal disturbanees are not infrequently seen but they are rarely severe enough to cause withdrawal of the drug, and then only temporarily. Oeeasionally, too, albuminuria and hæmaturia develop, with a deposit of P.A.S. erystals in the urine.

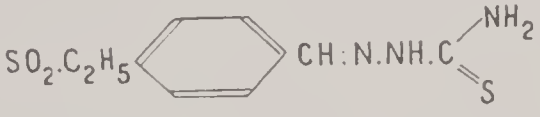
Delaude *et al.* (1949) have found that eultures of tuberele bacilli obtained from patients treated for long periods (157–251 days) with P.A.S. were highly resistant to P.A.S. Cultures from patients who reeeived P.A.S. in eombination with promin and streptomycin did not show this increase in resistance to P.A.S. *invitro*. The signifieance of these findings requires further investigation, including the implication that resistanee is less likely to develop when organisms are subjeeted to the aetion of two or more chemotherapeutic agents simultaneously.

Semicarbazones

These eompounds were first investigated by Domagk and his eollaborators who demonstrated their activity against the tubercle

bacillus both *in vitro* and *in vivo*. Their activity is not inhibited by p-aminobenzoic acid and they can be given orally, parenterally and locally (Domagk, 1947, 1948). Certain of these substances, of which the most important is p-acetamido-benzaldehyde thiosemicarbazone (TB1 698), have already been tried clinically, in combination with sulphathiazole, in the treatment of lupus and pulmonary tuberculosis, with apparently encouraging results (Moncorps and Kalkoff, 1947; Kuhlmann, 1948). Certain toxic effects were observed, such as initial gastro-intestinal disturbances, rashes, moderate anæmia, and in diabetics a marked rise in their requirement of insulin.*

Hoggarth *et al.* (1948) have studied about 100 derivatives and found that activity is confined to thiosemicarbazones of substituted benzaldehydes and heterocyclic aldehydes. The most active and least toxic of these compounds in mice is the thiosemicarbazone of p-ethylsulphonyl benzaldehyde (Compound 8388), of which the formula is shown below. The activity of this and several other compounds is higher than that of any other synthetic substance, including p-aminosalicylic acid.



Thiosemicarbazone derivative
(Compound 8388)

Spinks (1949) has devised spectrophotometric methods for estimating these compounds. He finds that compound 8388, when given to mice and rabbits, attains high blood concentrations and is only slowly eliminated. A high proportion is excreted unchanged in the urine. The persistence of this compound probably accounts for its high chemotherapeutic activity, since other thiosemicarbazones are more active *in vitro* but much more rapidly eliminated from the body. P-acetamidobenzaldehyde, the compound made by Domagk and tested clinically by his collaborators, lacks the persistence in the body shown by Compound 8388 (Spinks, 1949).

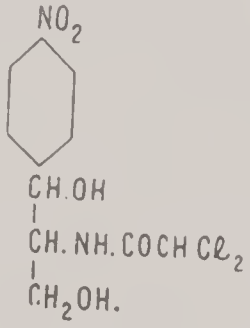
* The clinical results with TB1 (Conteben) are reviewed by Mertens, A., and Bunge, R., 1950 (*Amer. Rev. Tubere.* **61**, 20). A very interesting finding is that pulmonary tuberculosis with cavernous processes often responds well to oral administration of the drug, and that intracavernous treatment may be of value even in severe, progressive cavernous processes.

Chloramphenicol and Aureomycin

Many new antibiotics have been recently isolated, among which *chloramphenicol* and *aureomycin* deserve special mention, although they are not, as was at first hoped, active against true small viruses. This is still a problem that awaits solution. The properties of these two substances are reviewed by Schoenbach (1949).

Chloramphenicol (Chloromycetin)

Burkholder and his colleagues of Yale University (Ehrlich *et al.*, 1947) found in 1947 that an antibiotic substance was produced by a new strain of streptomyces, found in a soil sample collected near Caracas, Venezuela. The same streptomyces has incidentally been found more recently in a compost heap in Illinois (Gottlieb *et al.*, 1948). Agar cultures of this organism were found to inhibit adjacent inocula of a wide variety of gram positive and gram



Chloramphenicol
(chloromycetin)

negative organisms, and filtrates of deep cultures had the same antibacterial properties. A crystalline product was obtained which was called chloromycetin and is now also known as chloramphenicol. The chemical structure of the substance was rapidly determined, and it was found to be D-(-)-*threo*-1-p-nitrophenyl-2-dichloroacetamido-propane-1 : 3 diol. Parke Davis and Co. have given an account of its synthesis, by a team headed by Dr. Leon A. Sweet, in a paper read to the American Chemical Society on March 29, 1949, and the synthesis is described by Controulis *et al.* (1949). Raistrick (1949) also discussed its constitution. No doubt related compounds are at present being made and tested.

Chloramphenicol has been manufactured on a commercial scale and this product has been found effective experimentally and clinically (Smadel *et al.*, 1949).

Chloramphenicol is active in high dilutions against a number of gram positive and gram negative organisms (Smith *et al.*, 1948), but has only a moderate activity against M. tuberculosis. It is active against infections in mice with the *cholera vibrio* and this suggested its use in the treatment of human cholera and its prophylactic use during epidemics (Gauld *et al.*, 1949). By experiments on infected chick embryos and infected mice, chloramphenicol was shown to have chemotherapeutic activity against *rickettsial diseases* and *virus diseases of the psittacosis lymphgranuloma venereum* group, but it has no effect against the true viruses, such as Type A. influenza, St. Louis encephalitis and fixed rabies virus.

Chloramphenicol was isolated in a crystalline form by Bartz *et al.* (1947), who describe its physical and chemical properties. The substance is only slightly soluble in water, but soluble in propylene glycol. It is well absorbed when given orally to animals and man and rapidly metabolized, so that only about 10 per cent is excreted in the urine. Hence frequent administration is necessary to maintain a chemotherapeutic concentration. It can also be safely given intravenously, dissolved in an innocuous organic solvent, e.g. propylene glycol (Payne *et al.*, 1948). Its toxicity in laboratory animals is of the same order as that of streptomycin (Smith *et al.*, 1948). It can be assayed biologically by a method involving the inhibition of growth of Shigella paradysenteria (Ley *et al.*, 1948).

Ley *et al.* (1948) showed that when a dose of 2 grams is given to man, a blood concentration of about 20 μg./ml. is rapidly produced and the drug is detectable in the blood for more than eight hours. These authors also found that repeated doses of 0.2 gram did not produce a concentration detectable in the blood, but the urinary concentration was maintained at about 25 μg./ml. Smadel and co-workers (1948) also followed the blood and urine concentrations in patients suffering from typhus, and found that blood concentrations of the order of 5 μg./ml. could be maintained by the administration of 1 gram per day, given as 0.2 grams at four hourly intervals. Glazko *et al.* (1949) have followed the distribution of chloramphenicol in rats and dogs and found high concentrations in the kidney and liver, with progressively lower concentrations in the lung, spleen, heart, muscle and brain. Incubation of the drug with rat liver slices results in a loss of activity. In man less than 10 per cent of the drug is excreted in

the urine unchanged but most of the remainder is excreted in the form of an inactive nitro compound.

The clinical investigation of chloramphenicol is still in its early stages and only some indication of its value can at present be

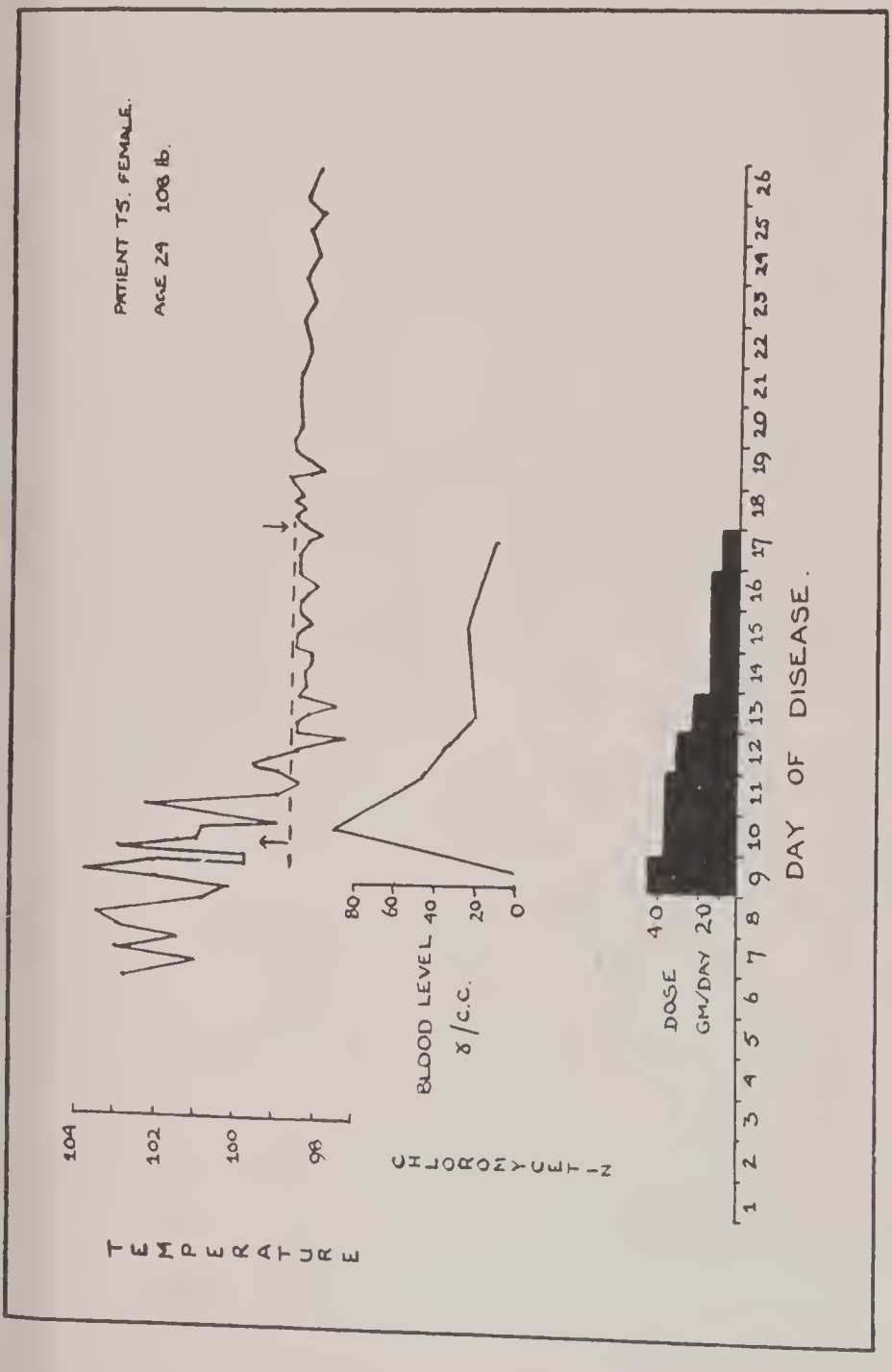


FIG. 17
Showing the effect of treatment with chloramphenicol in typhoid fever.
(After Woodward *et al.*, 1948)

given. It is clearly highly effective in the treatment of *scrub typhus* and the possibilities of its use in the prophylaxis of this disease are also being investigated (Smadel *et al.*, 1948 ; *Lancet*, 1949). It is also rapidly effective in the treatment of *epidemic typhus* (Payne *et al.*, 1948 ; Smadel *et al.*, 1948), especially when given by slow intravenous injection, dissolved in propylene glycol, and in *rocky mountain spotted fever* (Pincoffs *et al.*, 1948). It is also of value in the treatment of *typhoid fever* (Woodward *et al.*, 1948), and should be administered for more than eight days to acutely ill patients if relapses are to be avoided ; there appears to be little advantage in continuing treatment for more than 14 days (Smadel *et al.*, 1949). According to the results of El Borolossy and Buttle (1949) on experimental *S. typhi* infections in mice, a combination of chloramphenicol and sulphadiazine might provide an even more satisfactory treatment. Encouraging results have been obtained with chloramphenicol in the treatment of *infantile gastro-enteritis* (Rogers *et al.*, 1949) and *paratyphoid B fever* (Curtin, 1949). Interesting findings when administered in *early syphilis* in man have also been reported (Romansky *et al.*, 1949).*

Aureomycin

This is also produced by a mould of the streptomyces group (streptomyces aureofaciens) and was isolated by workers at the Lederle Laboratories. It has been obtained in a highly pure crystalline form and is a golden yellow substance. It is soluble in water and quite stable in acid solutions (pH 4.0). Neutral or alkaline solutions rapidly lose their activity. Many findings on aureomycin are collected in a number of the Annals of the New York Academy of Sciences (1948, **51**, 175–345).

The drug is active against a number of gram positive and gram negative organisms (Paine *et al.*, 1948) and, according to Finland *et al.* (1948), it is particularly effective in experimental infections with *rickettsias* and with *viruses of the psittacosis lymphgranuloma venereum* group. It is also active against *spirochaetes* : Heilman (1948) found it effective in the treatment of experimental relapsing fever in mice and in experimental leptospirosis in

* Chloramphenicol has been found of value in whooping cough. It is also capable of sterilizing the upper respiratory tract, but the implications of this remarkable finding remain to be investigated (Gray, J. D., 1950, *Lancet*, i, 150).

hamsters, and O'Leary *et al.* (1948) demonstrated some anti-spirochetal activity in *primary syphilis* in man. Although aureomycin has some action on tubercle bacilli *in vitro*, the results of treatment of tuberculosis in guinea pigs and man have been negative (Steenken and Wolinsky, 1949; Steinbach *et al.*, 1949).

Aureomycin is inactivated too rapidly *in vitro* to determine bacteriostatic concentrations by a 24 hour serial dilution method as used for penicillin, and investigators have used a dilution method with *Bacillus cereus* as the test organism, the results being read after four hours of incubation at 37°C.; this is based on the test first described by Dornbush and Pelcak (1948). As aureomycin is so unstable in body fluids, Herrell and Heilman (1949) store their biological material in a frozen state in a chamber refrigerated with solid CO_2.

Aureomycin is well absorbed when given orally and rapidly appears in the urine. Some 10–15 per cent of the dose given is excreted by the kidneys. When the drug is given orally in single doses of 0.75–1 gram the maximum serum concentration (up to 4 μg./ml.) is reached after some 3–6 hours and this is maintained for several more hours. Some activity may be detected in the serum for 24 hours or even more. With continued administration serum levels up to 20 μg./ml. may be found. The results suggest that six hourly oral doses of 10 mg. per kg. should be adequate for therapeutic purposes. Following intramuscular administration the maximum is reached more rapidly, in about one hour. The drug diffuses into many body tissues and fluids, including the cerebrospinal fluid, in which it is present in therapeutically effective concentrations. It passes through the placental barrier, and is concentrated in the liver and excreted in the bile (Lepper *et al.*, 1949; Brainerd *et al.*, 1949; Herrell and Heilman, 1949). The drug has a low toxicity and produces almost no side reactions in animals. In man nausea and occasional diarrhoea have been observed, as well as some rise in temperature (Spink *et al.*, 1948).

Clinical Use

It is too early yet to estimate the value of the drug clinically. Good results have been obtained in the treatment of a rickettsial disease, *rocky mountain spotted fever* (Ross *et al.*, 1948), in *brucellosis* due to brucella melitensis, suis and abortus (Spink *et al.*, 1948; Debono, 1949; Bryer *et al.*, 1949) and in *tularaemia*. In

this last condition the effect is apparently as good as that produced by streptomycin (Woodward *et al.*, 1949). Other conditions are also being investigated including a variety of diseases due to bacterial infections (Finland *et al.*, 1948; Collins *et al.*, 1948; Braley and Sanders, 1948), and also *primary atypical non-bacterial pneumonia* (Schoenbach and Bryer, 1949). Rutenburg and Schweinburg (1949) report good results in *urinary infections* with gram negative organisms which were refractory to previous treatment with other antibiotics. The wider and successful use of the drug in urinary tract infections is described by Collins and Finland (1949); Willcox and Findlay (1949) found aureomycin effective in *gonoccocal* and *non-speeific urethritis*. Preliminary results suggest that the drug may prove of value in the treatment of *amoebiasis* (McVay *et al.*, 1949), and of anthrax (*Brit. med. J.*, 1949).

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CHAPTER 8

ANTIMALARIAL DRUGS

The last war gave a great impetus to research in this field, as several campaigns were fought in areas where malaria was prevalent, and armies were more than once almost immobilized because of ineffective control of the disease. This was due, to a great extent, to the fact that the supply of quinine was cut off by the Japanese invasion of the East Indies, but also to a lack of knowledge of the proper use of alternative substances. The advances which have since been made can be described under four headings, namely :—

(1) Methods of large scale manufacture of drugs already known. Mepacrine and pamaquin were such drugs and had previously been manufactured by the Germans, and much work and skill was necessary to solve the problem of their large scale preparation. This was essentially a chemical problem.

(2) The discovery of new and more effective remedies. The most important drugs so found were proguanil (paludrine), pentaquin and chloroquine. Their discovery resulted from the collaboration of chemists and pharmacologists.

(3) The more effective use of all the drugs available in the prevention and treatment of malaria. This involved large scale clinical experiments and also some beautifully controlled experiments on the human subject. Clinicians and parasitologists were essentially responsible for this work. The trials performed on volunteers at a penitentiary in U.S.A. are described by Alving *et al.* (1948), and experiments carried out by Fairley and his colleagues will be referred to in the section on proguanil.

(4) The discovery of new and important information concerning the life cycle of the malarial parasite, made by parasitologists in Great Britain. This will certainly contribute to the understanding of the action of different drugs at various stages of the disease.

There has also been much work on the metabolism of the malarial parasite and the effect of various drugs on this, but the significance of the findings is not yet clear (Marshall, 1948).

Mackerras and Ercole (1949) conclude that quinine and mepacrine appear to act by inhibiting the breakdown of hæmoglobin which occurs in the presence of the parasites. These drugs have no action on parasites whose growth is independent of this protein. Pamaquin acts as a general protoplasmic poison, its action being independent of the substrate used for metabolism. Mature gametocytes are unharmed by quinine and mepacrine, but are very susceptible to the action of pamaquin. Quinine does, however, inhibit the growth of young gametocytes, whereas mepacrine affects their metabolism in such a way that they appear unpigmented or with only minute amounts of pigment. These unpigmented forms are capable of infecting mosquitoes.

The Life Cycle of the Malarial Parasite

As is well known, this can be divided into two phases, i.e. one during which the parasite is in the mosquito, and another during which it undergoes various changes in man (or other hosts, such as the monkey or a bird). The mosquito, by its bite, transfers the parasite to man in the form of the sporozoite and for many years it was believed, as Schaudinn had described it in 1902, that the sporozoite penetrates into the red cell and then undergoes its life cycle to trophozoite, schizont, merozoites and so on. No one had, however, actually been able to confirm Schaudinn's observation, and there were, in addition, certain findings on the action of quinine which suggested that the sporozoites differed fundamentally in their properties from the parasites present in the red cells. These observations were made on patients given malarial therapy for general paresis : If a patient is inoculated with blood containing malarial parasites and the subsequent infection treated with quinine, he is easily and permanently cured. Hence the parasites at that stage are highly susceptible to the chemotherapeutic action of quinine. But if a patient is inoculated with sporozoites and then treated with quinine, this does not prevent the attack of malaria ; and the drug given at a later stage, when the typical attack has developed, will only produce a temporary remission of symptoms and not prevent a relapse. Hence sporozoites, and some forms descended from them, are resistant to the action of quinine. It was also found that if patients are injected intravenously with large amounts of infected corpuscles, the attack develops almost immediately ; if sporozoites are injected,

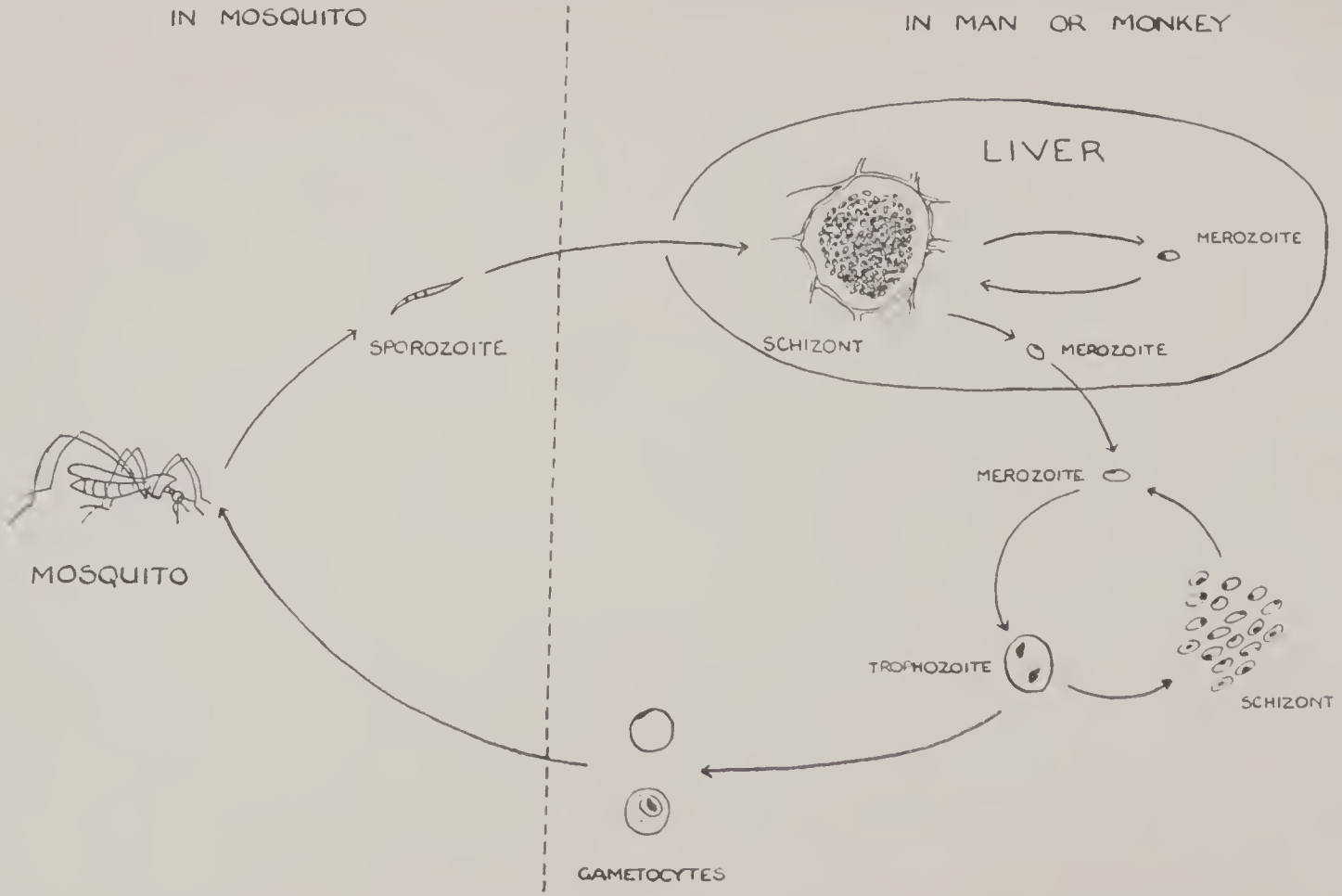

IN MOSQUITO
IN MAN OR MONKEY
LIVER
EXOERYTHROCYTIC
CYCLE
MEROZOITE
SPOROZOITE
SCHIZONT
MEROZOITE
MEROZOITE
ERYTHROCYTIC
CYCLE
MOSQUITO
TROPHOZOITE
SCHIZONT
GAMETOCYTES

FIG. 18

even in large numbers, there is an incubation period of not less than five days in malignant tertian, and of about nine days in benign tertian malaria. During this period the blood of such patients is not capable of infecting other subjects.

These observations can best be explained by the assumption made by a number of observers, that the sporozoites undergo changes outside the blood for a period of some days; from such pre-erythrocytic forms parasites then pass into the blood and undergo their classical changes. Exo-erythrocytic forms were described in bird malaria by James and Tate (1937) and by other investigators; this was followed by the demonstration that in bird malaria, sporozoites introduced into the body (by mosquitoes or by injection) enter the macrophages of the skin and other organs and pass through several generations as exo-erythrocytic forms before the corpuscles become infected (Reichenow and Mudrow, 1943; Huff and Coulston, 1944).

That there is an exo-erythrocytic cycle in malaria in man (*P. vivax*) and in the monkey (*P. cynomolgi*) is the important contribution very recently made by Shortt and Garnham (1948). They infected monkeys with large numbers of sporozoites of *P. cynomolgi* by injecting them with ground up mosquitoes. The animals so infected were examined in two ways. Either a special day during the incubation period was selected and the monkey was killed for post-mortem examination of all the organs, or a biopsy was performed on the liver of the anæsthetized animal. This could be done frequently and the infection could thus be followed in the same animals from the early stages until the establishment of blood infection. Shortt and Garnham found that large parasites, which they name schizonts, developed in the liver and that these ultimately ruptured and released merozoites. The biopsy technique was used to demonstrate the presence of schizonts of *P. vivax* in the liver of man. In addition to biting by large numbers of infected mosquitoes, infected salivary glands of mosquitoes were injected intravenously into a patient, and seven days later a biopsy specimen of the liver was taken. This also showed the typical schizonts, in some cases with release of merozoites (see Fig. 19). In order to determine whether such pre-erythrocytic forms are only temporarily present, or whether they remain in the animal after the blood infection has set in, Shortt and Garnham (1948) examined the liver of a monkey

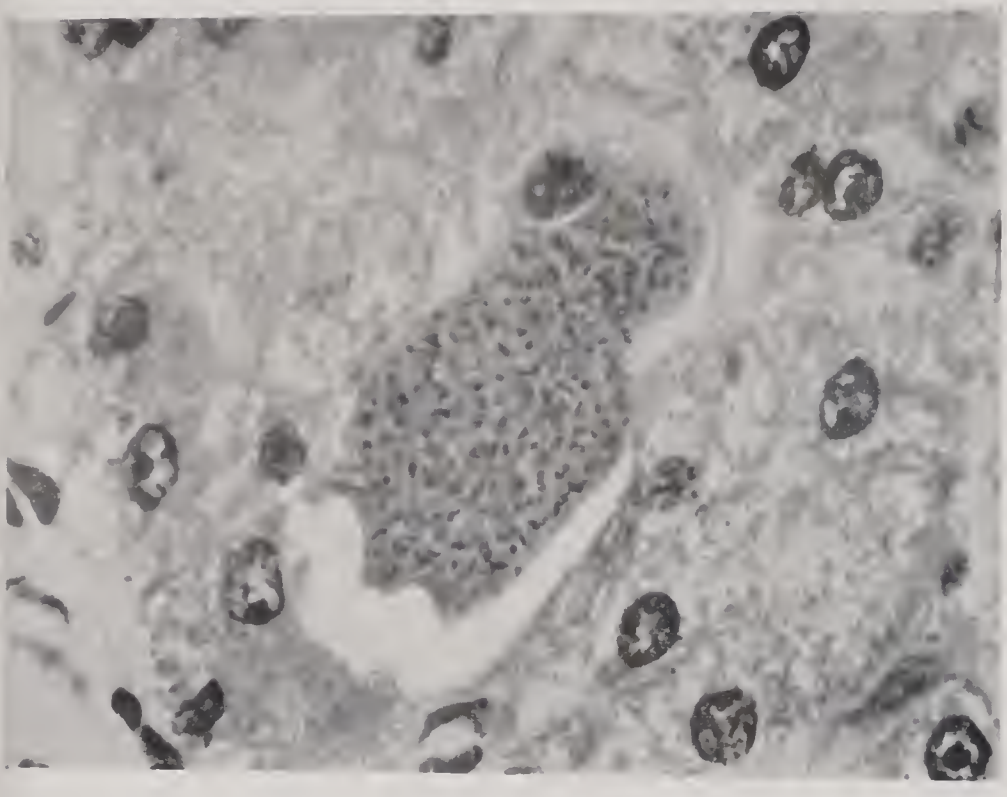


FIG. 19

Schizont of *P. vivax* in human liver $\times 1000$. Note at upper end the nucleus of the liver cell containing the parasite, which has itself shrunk away to some extent from the surrounding liver tissue. Numerous particles of chromatin are to be seen inside the parasite.

(Very kindly supplied by Col. H. E. Shortt.)

[*To face page* 190.

three and a half months after a sporozoite-induced infection. The infection was latent at that stage and a piece of liver was removed for examination. They chose that particular time for the operation as they believed that a relapse would soon occur, and in actual fact early ring forms were found in the blood on the following day. The liver biopsy specimen contained typical exo-erythrocytic schizonts.

More recently still the existence of a pre-erythrocytic stage in the life cycle of *P. falciparum* has been demonstrated in an experiment on Mr. C. H. Howard "who not only cheerfully, but on his own initiative, offered himself for this experiment" (Shortt *et al.*, 1949).

From all their observations Shortt and Garnham conclude that "The inoculation of sporozoites by the infected mosquito is followed by a pre-erythrocytic development in the parenchymal cells of the liver, with the ultimate production of merozoites. Many of these enter the erythrocytes to produce a parasitæmia and a clinical attack of malaria. Other merozoites enter normal liver cells and repeat the process of exo-erythrocytic schizogony. This latter process repeats itself indefinitely, irrespective of whether the erythrocytic cycle is present or is in abeyance as the result of antimalarial treatment, or a naturally acquired active immunity. The active immunity is primarily operative against the erythrocytic parasites, but also destroys those merozoites liberated by the exo-erythrocytic schizonts which are destined to enter red cells. Those which enter liver cells to maintain the exo-erythrocytic cycle are protected from this immunity by their intracellular position outside the circulating blood. If, for any reason, the active immunity of the host is impaired, it no longer operates against the merozoites, destined to start the erythrocytic cycle, and these enter the blood cells and initiate a clinical relapse."

The tissue forms of the parasites ultimately do die out, the time required varying with the Plasmodium responsible. In the case of *P. falciparum* they probably die soon after the infection has developed in the blood corpuscles. In the case of *P. vivax* they appear to persist for 1–3 years, giving rise at intervals to the relapses typical of benign tertian malaria, while the tissue forms of quartan malaria persist for a much longer period, perhaps up to 20 years.

The Discovery of New Drugs

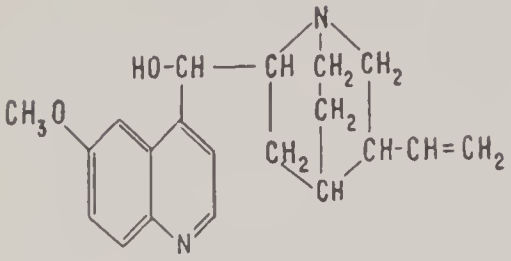
These findings emphasize that what is needed in the treatment of malaria is a drug, or combination of drugs, which will produce a chemotherapeutic action against the exo-erythrocytic forms, and not merely against the blood forms of the parasite. This would lead to the eradication of the schizonts from the liver, thus preventing the development of the infection when given as a prophylactic, and avoiding relapses when given to cure the clinically established condition. In addition, drugs effective against the sexual forms would also be useful since they would prevent the dissemination of the disease. Lastly, of course, measures which lead to the eradication of the mosquitoes from an area are the most certain way of ensuring that malaria is no longer spread.

In practice drugs are used to produce three main effects, viz. (1) Prophylaxis, i.e. to kill the parasites before they can produce an infection, (2) Suppressant: for this purpose the drugs are given over long periods and though infection may occur its clinical manifestations are kept in abeyance, and (3) Curative, i.e. to eradicate completely an established infection.

In the search for new antimalarials which was carried out during the war, and which is still proceeding, many thousands of drugs were tested on laboratory animals. Some of these investigations are surveyed in an American monograph edited by Wiselogle (1946). Many tests were carried out in bird malaria. For example, in the investigations which resulted in the discovery of proguanil, the initial tests on new compounds were performed on young chicks infected with *P. gallinaceum*; other hosts, infected with other strains of parasites, were used to distinguish between drugs thought to act in different ways (Curd *et al.*, 1945).

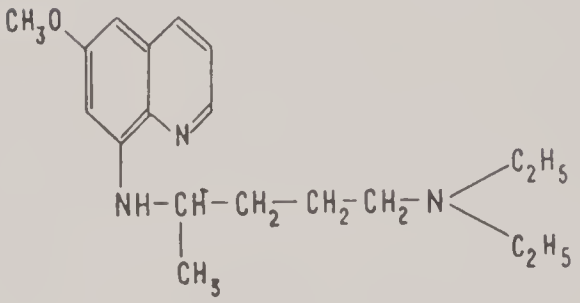
It has recently been shown that *P. cynomolgi* infections in monkeys react to drugs in a manner very similar to *P. vivax* infections in man, and it seems possible that rhesus monkeys may be valuable in a systematic search for chemotherapeutic agents which will cure human infections with *P. vivax* (Genther *et al.*, 1948).

Antimalarial Drugs

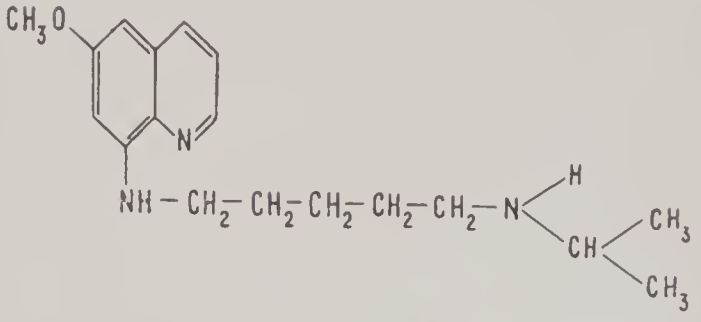


Quinoline group Quinuclidine group

Quinine

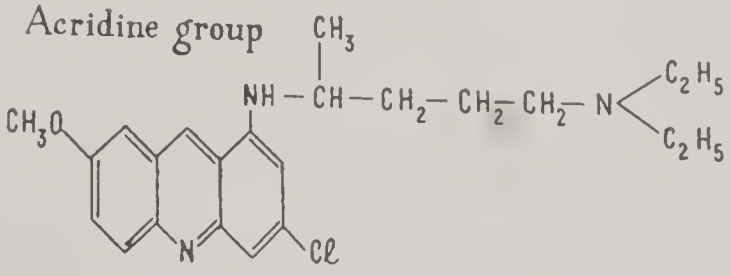


Pamaquin

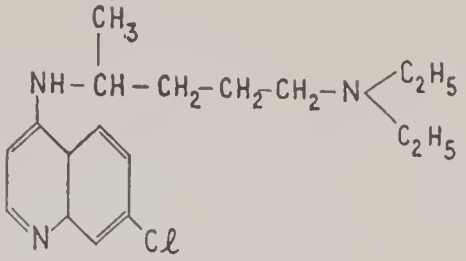


Pentaquine

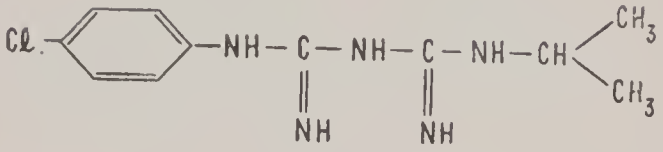
Acridine group



Mepacrine



Chloroquine



Proguanil

Proguanil (Paludrine)

Before the war three drugs were in use in the treatment of malaria, namely quinine, mepacrine and pamaquin. Many thousands of compounds have been made which are essentially modifications of these known active substances. Proguanil is, however, exceptional in that it represents an antimalarial with an entirely new chemical structure. The story of its discovery in the I.C.I. laboratories, as told by Rose (1946), makes fascinating reading. Starting with simple compounds containing the pyrimidine nucleus, which is present in so many substances of physiological importance, they arrived at proguanil by a combination of bold planning, patient trials and, most important, inspired guesswork. Much time was spent trying to produce a substance similar in constitution to riboflavine, and possibly able to act on the parasite by a mechanism of substrate competition, but this did not lead to the discovery of any very active compounds. An important advance was made when it was found that the activity of the compounds under investigation was dependent on the middle part of the molecule consisting of alternate carbon and nitrogen atoms, and this led to the biguanide type of structure and ultimately to proguanil. A great advantage of proguanil is that it can be easily and cheaply prepared in large quantities, and this is the first time that a synthetic drug of this type has become available.

Actions

Proguanil inhibits the development of the blood forms of all species of malarial parasites so far examined in *birds* (Curd *et al.*, 1945). The effects of the drug in *man* were extensively investigated by a team under the direction of Fairley at Cairns, Australia, and are described by Fairley (1946). This work involved the experimental infection of volunteers with both *P. vivax* and *P. falciparum* malaria and their treatment at various stages following infection. Soldiers evacuated from New Guinea with vivax or falciparum malaria were also treated. It was found that proguanil is a *causal prophylactic* against mosquito induced falciparum malaria but only a *partial prophylactic* against vivax malaria: in the latter case the exo-erythrocytic forms are only inhibited but not destroyed, and after the administration of the drug has ceased, the surviving forms commence schizogony with eventual production of asexual erythrocytic parasites and overt malaria. The drug in quite small doses produces *radical cure* of falciparum malaria, but even large doses fail to achieve this in the benign tertian variety. Working with an African strain of *P. falciparum*, Covell *et al.* (1949) found, as did Fairley, that proguanil acted as a true causal prophylactic. The drug, however, failed to effect radical cure of infections with this strain, a finding in marked contrast to the results of Fairley on the New Guinea strain of *P. falciparum*.

Proguanil does not act on the sexual forms, i.e. it has *no gametocidal properties* but, if it is present in human blood containing gametocytes of either *P. vivax* or *P. falciparum* at the time of ingestion by mosquitoes, the sexual development of these parasites in the insect host is inhibited so that the sporozoites are not formed. This is a very important effect since it should materially decrease the chances of infection in areas where the drug may be extensively used in the suppression of the disease.

Drug Resistance

Williamson and Lourie (1947) have shown that *P. gallinaceum* can develop a high degree of resistance to proguanil, which is transmissible through the mosquito (*Aëdes ægypti*). No such resistance was obtained following intensive and prolonged treatment with quinine, mepacrine, a pyrimidine precursor of proguanil, or sulphadiazine. The development of resistance to sulphadiazine has since been described (Bishop and McConnachie, 1948). Schmidt

and his co-workers (1949) have found that a high degree of proguanil resistance can be developed when infections with *P. cynomolgi* are treated with successively increasing doses of the drug ; this resistance too can be transferred through the mosquito without loss. The possible development of *proguanil resistance in human malaria* is obviously a matter that requires investigation. The experiments of Seaton and Lourie (1949) in man suggest that the danger of producing a serious degree of resistance in a strain of *P. vivax* by the ordinary use of proguanil in clinical practice is very slight, if not negligible, but Cooper and his co-workers (personal communication from Dr. W. Clark Cooper) have found that resistance can easily be induced in patients experimentally inoculated with the Chesson strain of *P. vivax*, by giving doses below those required for complete eradication. This resistance can be transferred through the mosquito. Attempts to produce resistance to chloroquine by similar techniques have not proved successful. Dr. Cooper concludes "that chlorguanide (i.e. proguanil) resistance should not offer much hazard to the individual patient in a mildly endemic zone. On the other hand, use of the drug for mass therapy and prophylaxis in a hyperendemic area would seem to provide excellent opportunities for acquired resistance." He believes that resistant strains would become widespread under such conditions.*

Absorption and Fate

In *animals* proguanil is fairly rapidly absorbed from the intestine and about 70–90 per cent of the dose is absorbed (Schmidt *et al.*, 1947). The blood levels produced are relatively low, but the drug is concentrated in certain organs, including the lungs, liver, spleen and kidneys. The concentrations in red cells are 4–6 times as great as those in plasma, and white cells probably contain 10–100 times as much as plasma. Prognanil is excreted mainly in the urine but some reaches the large intestine (Spinks, 1947).

The fate of the drug in *man* has been investigated by Macgraith *et al.* (1946). They found that it is quite rapidly absorbed from the intestine ; the maximum plasma concentration is reached some four hours after ingestion and then falls to half in about twelve hours. They also determined the plasma concentrations produced

* The occurrence of proguanil-resistant falciparum malaria in Malaya has since been described by Edeson, J. F. B., and Field, J. W. (1950, *Brit. med. J.*, i, 147).

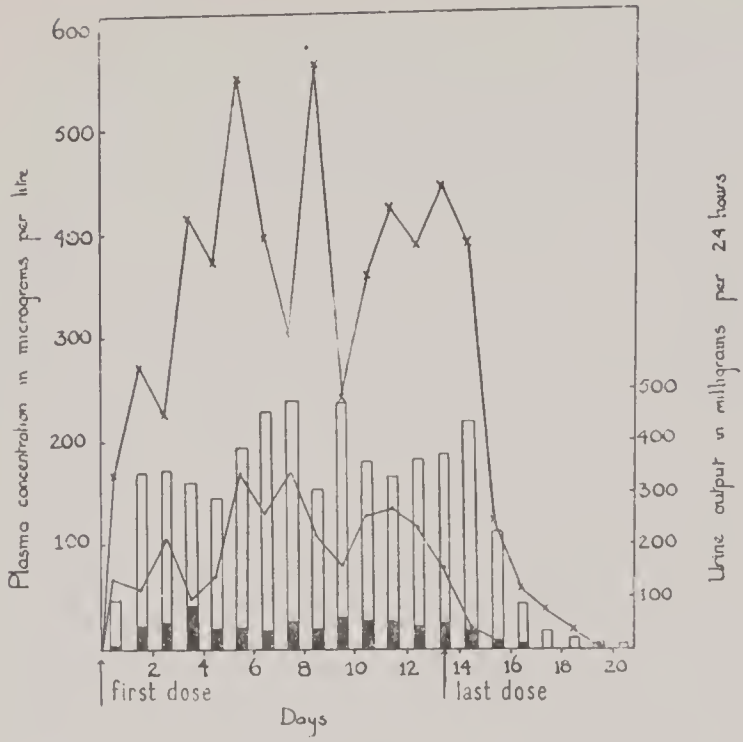


FIG. 20

Showing the plasma concentrations and urinary excretion of proguanil during 14 day courses of 500 mg. and 50 mg. twice a day.

Plasma concentration on 500 mg. proguanil b.d.

Plasma concentration on 50 mg. proguanil b.d.

Urinary excretion on 500 mg. proguanil b.d.

Urinary excretion on 50 mg. proguanil b.d.

(After Maegraith *et al.*, 1946.)

by the continued administration of the drug (see Fig. 20). The blood concentrations are about three times those found in the plasma, and about 75 per cent of the plasma proguanil content is protein-bound. The distribution of the drug in organs is very similar to that found in animals, the highest concentration being found in the kidneys. Of the total dose given, 40–60 per cent is excreted by the kidneys, and about 10 per cent in the stools.

Toxicity

The toxicity of proguanil varies greatly among different species of animals (Schmidt *et al.*, 1947 ; Butler *et al.*, 1947). In man the difference between the effective therapeutic dose and the toxic dose is high and definitely exceeds that possessed by any other antimalarial drug. Fairley (1946) found that with the usual dosage, such as 300 mg. daily for 10–21 days, significant toxic symptoms were not observed. In doses of 1 G. per day given to volunteers with overt malaria, toxic effects were seen, but they were not serious and could be relieved by diminishing the daily dose or by cessation of therapy for 1–2 days. The chief toxic effects are vomiting, and the presence of red cells and casts in the urine. Gross hæmaturia occasionally develops. No toxic effects were obtained when the drug was given as a suppressant over periods of three months (100 mg. per day) or six months (100 mg. twice weekly). Later reports, however, suggest that the prolonged administration of proguanil for prophylactic purposes may produce loss of appetite, reduction of weight and lack of energy (Annotation, 1949) and similar effects have also been observed in experimental animals (Schmidt *et al.*, 1947). It has been shown that proguanil inhibits the gastric secretion in animals and man (Burn and Vane, 1948 ; Vane *et al.*, 1948) and this may possibly explain these effects.

Mode of Action

Curd and Rose (1946) have made the interesting suggestion that the action of proguanil is due to an interference with the porphyrin metabolism of enzyme systems of the malarial parasite. The drug forms a copper derivative in which two molecules of proguanil are combined with one atom of copper. This complex is rather similar in structure to the naturally occurring porphyrin pigments and might thus compete with them at some stage of their metabolism in the parasite.

It has been shown that proguanil has no action *in vitro* on the exo-erythrocytic forms of *P. gallinaceum* or on the endo-erythrocytic forms of *P. cynomolgi*. The serum of animals which have recently received proguanil, or proguanil which has been incubated with minced liver, has a marked action *in vitro* (Hawking and Perry, 1948). This suggests that proguanil itself is not active against plasmodia but that it can somehow be modified in the body, perhaps

by the liver, so that it becomes actively plasmodicidal. This conclusion is also supported by the investigations of Madinaveitia and Raventós (1949). They found that antimalarial substances inhibit certain effects of adenosine, which may mean that antimalarial action in some way involves interference with a process, essential to the parasite, in which adenosine or a derivative thereof is concerned. Proguanil itself does not act in this respect like the other antimalarials, but the blood of guinea pigs treated with proguanil does antagonize the action of adenosine; this is taken as evidence that proguanil is metabolized to a compound active against the malarial parasite.

Pentaquine and Pamaquin

These drugs are similar in chemical composition and actions and are derivatives of aminoquinoline, with a methoxy group (CH_3O) in position 8 (see formulæ on p. 193). *Pamaquin* has actions on the gametocytes and, in combination with quinine, is highly effective in the treatment of malaria, but its toxicity has precluded its general use for this purpose, though Monk (1948) believes this has been greatly exaggerated. *Pentaquine* is less toxic than pamaquin and can thus be used more safely in combination with quinine in the treatment of malaria. A still newer compound, *isopentaquine*, is apparently even less toxic than pentaquine (Alving, 1948).

Absorption and Fate

Pentaquine is rapidly and completely absorbed from the intestine of experimental animals. Peak plasma levels are obtained between one and a half and two hours after administration and decline steadily thereafter. With doses of 30 mg. per day the average plasma concentration is 3.4 μg./100 ml., ranging from 2.5 to 4.8 μg. /100 ml. (Alving *et al.*, 1948). The concentrations which remain at the end of 6–8 hours are barely detectable with the method available (i.e. they must be less than 2 μg./100 ml. of plasma). Not more than 15 per cent of the amount ingested can be recovered from the urine. Pentaquine is rapidly metabolized and, unlike mepacrine and chloroquine, is not concentrated in the tissues even with prolonged treatment. Hence the metabolism of pentaquine is very similar to that of pamaquin, the only

significant difference being that the plasma levels of pentaquine are maintained somewhat longer than those of pamaquin.

The fate of the drugs in man is very similar. Plasma levels of pentaquine can be fairly well maintained by administering the drug on a four hour schedule throughout the day and night. The concentration of the drug in the plasma is usually slightly lower than that which results from the administration of an equivalent dose of pamaquin (Loeb, 1946).

Actions

Pentaquine, like pamaquin and several other 8-methoxy-aminoquinolines, exerts a definite prophylactic effect in *P. vivax* infections of the Chesson strain. The strain of the infection is usually stated in experimental work, as different strains vary widely in their response to drugs. The Chesson strain, obtained in the Pacific, is one which is comparatively resistant to the action of drugs. The effects of pentaquine and related substances are probably exerted on the pre-erythrocytic stages of the malarial parasite. The doses which have to be used, however, are near the maximum amounts tolerated, so that these drugs cannot be used as causal prophylactics, but the fact that they have an action on the exo-erythrocytic forms probably help to explain why a combination of pentaquine, or pamaquin, with quinine is so effective in the treatment of *P. vivax* infections and in the prevention of relapses, which are presumably due to the survival of exo-erythrocytic forms in the liver. Of interest too, is the finding that in those subjects whose primary attacks were not prevented by prophylactic treatment with the 8-aminoquinolines, there is evidence that the disease is so altered by the prophylactic therapy that it is rendered susceptible to cure by drug regimes usually ineffective in preventing relapse (Jones *et al.*, 1948). The curative effects of pentaquine in *P. vivax* infections (Chesson strain) are described by Alving *et al.* (1948). Even when given alone the drug has some action in preventing relapses, but this is greatly enhanced by combining it with quinine.

Toxicity

In animals the toxicity of pentaquine is about one half to one quarter that of pamaquin. The ratio varies in different species and with different methods of administration. The toxicities of

both drugs are increased two to four times by the concurrent administration of quinine (Loeb, 1946). These effects have been studied quantitatively by Chen and Geiling (1947).

When given in man in doses of 60 mg. per day (of the base) toxic effects severe enough to cause discontinuance of the treatment are rarely seen. The toxic effects are similar to those produced by pamaquin, and consist of abdominal discomfort and pain, anorexia and occasionally transient weakness, headache and diarrhoea. Alving *et al.* (1948), who made these observations, also found that the plasma concentration with this dose varied from 29 to 49 μg. per litre and was raised when proguanil was given at the same time, though the therapeutic effectiveness was not increased. When higher doses (120 to 180 mg., i.e. two or three times the therapeutic dose) were given to volunteers, the toxic symptoms were severe and included, in addition to those mentioned above, methæmoglobinæmia and the production of long persistent postural hypotension and syncope (Craige *et al.*, 1948). The toxicity of pentaquine is definitely lower than that of pamaquin or other related aminoquinolines (Alving *et al.*, 1948).

In the treatment of *P. vivax* malaria a daily dose of 60 mg. of pentaquine base (equivalent to 80 mg. of the diphosphate, which is the compound usually used) and 2 G. of quinine are recommended. The drugs should be given concurrently in divided doses every four hours for 14 days and this should produce radical cure of severe infections. It is emphasized that pentaquine should be administered only under close medical supervision, preferably in hospital. Monk (1948) states that 60 mg. of the drug per day is too high a dose to be given as a routine without further extensive trials.

Chloroquine and Mepacrine

These two substances have similar actions on the erythrocytic forms of *P. vivax* and *P. falciparum*. They are ineffective against the exo-erythrocytic forms of *P. vivax*. Overt attacks of malaria are quickly aborted by proper treatment. If they are due to *P. falciparum*, a radical cure of the infection results, but such is not the case in vivax infections. Overt attacks are cured but a relatively large proportion of treated patients suffer relapse. Studies of the antimalarial activity of chloroquine against well standardized strains of *P. vivax* and *P. falciparum* have shown that

its activity is about three times that of mepacrine (Loeb, 1946). Chloroquine has the additional advantage that it is colourless and hence does not stain the skin.

Mepacrine

This was discovered by Schulemann in 1930 and used quite extensively before the war, but much work had to be done on it before it could be used effectively on a large scale during the war. It was then extensively used in the control of malaria in many theatres of operation.

Mepacrine is readily absorbed from the intestine, but only a small fraction of the daily dose is excreted in the urine. Substances derived from mepacrine have also been identified in the urine (Hammick and Mason, 1945). Some of the drug is found in the faeces, even when it is administered intravenously (Army Malaria Research Unit, 1946). Much of the drug is deposited in tissues ; for example, the concentration of mepacrine in the liver and spleen may be 10,000 to 20,000 times as high as that found in the plasma. Experiments have been performed to determine the minimum plasma concentration of mepacrine necessary to relieve the symptoms and remove the parasitæmia in malaria. In the case of *P. vivax* infections 30 μg. per litre maintained for four days is sufficient, but 50 μg. per litre maintained for six days is necessary for *P. falciparum* malaria (Blanchard, 1947). Oral doses of the drug needed to maintain plasma concentrations above these critical levels were worked out, and their use has given very satisfactory results in the treatment of malaria. Ellerbrook *et al.* (1945) gave 2.8 G. of mepacrine in one week to patients suffering from malaria and found an average fasting plasma concentration of 41 to 52 μg. per litre. After cessation of treatment the levels decreased slowly, so that by four weeks after the beginning of treatment the average fasting concentration was still 8 μg. per litre (see Fig. 21). The therapeutic results with this dose were satisfactory. The plasma concentrations were determined by the method of Masen (1943), which is sensitive and sufficiently specific to differentiate between mepacrine and its fluorescent derivatives. Macgraith and his co-workers (1946) found that, on a therapeutic course of 4.6 grams of mepacrine over twelve days, the mean plasma concentration was 65.2 μg. per litre, with a range of 132-20 μg. per litre. Up to 80 per cent of the mepacrine in

whole blood is in the white cell layer (Army Malaria Research Unit, 1946).

Chloroquine

This was discovered and patented in Germany before the war but was not then subjected to intensive biological testing or adequate clinical study.

Chloroquine, used as the diphosphate, is rapidly and completely or nearly completely, absorbed from the intestine. Some 10–20 per cent of it is slowly excreted unchanged in the urine, though its excretion is somewhat more rapid than that of mepacrine. Considerable amounts are deposited in certain organs and tissues, and it is concentrated in nucleated cells, particularly those of the

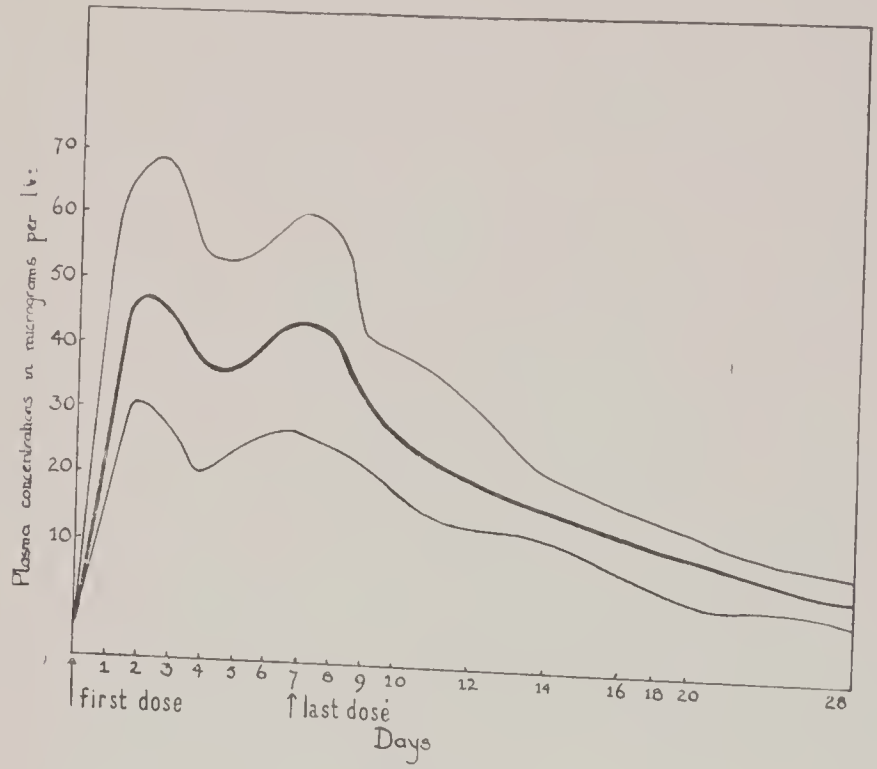


FIG. 21

Fasting plasma mepacrine concentrations on a large group of patients who received 2.8 G. of mepacrine hydrochloride in one week. The heavy line indicates average values, and the fine lines indicate one standard deviation (i.e. includes about two thirds of all values).

(After Ellerbrook *et al.*, 1945.)

liver, spleen, kidneys and lungs. These organs contain from 200 to 500 times the amounts present in the plasma. The drug is also concentrated to a considerable extent in leucocytes. Hence its pattern of distribution is similar to that of mepacrine. Chloroquine is metabolized in the body but this occurs slowly and it may be detected in body tissues for more than a week after cessation of its administration (Loeb, 1946).

There is little difference in the toxicity of chloroquine and mepacrine in animals. In man no serious toxic effects have been seen, and only very occasionally, usually with doses higher than those necessary for either treatment or suppression, have individual subjects refused to continue drug administration because of unpleasant symptoms. The toxic effects include mild and transient headache, visual disturbances, pruritus and gastrointestinal complaints (Loeb, 1946). Of thirty volunteers who received chloroquine for one year in a dosage in excess of that required for antimalarial suppression, two developed a lichen-like eruption similar to the rash occasionally caused by mepacrine. The eruption promptly disappeared when the drug was discontinued, and did not appear again when these two patients were given chloroquine at a later period (Craige *et al.*, 1948).

The Council on Pharmacy and Chemistry (1948) recommend the following dosage. For *treatment* of acute *P. vivax* and *P. falciparum* infections, an initial dose of 1 G. followed by an additional 0.5 G. after 6–8 hours and a single dose of 0.5 G. on each of two consecutive days (total of 2.5 G. in three days). For *suppression* of *P. vivax* malaria, a weekly dose of 0.5 G. given at exactly seven days interval is recommended.

Comparative Value of Drugs in Malaria

The malarial parasite in the human host can be affected by drugs at three stages of its cycle and the drugs which produce an action at these different stages of *P. vivax* are shown in Table 5. The modern therapy of benign tertian malaria is most comprehensively reviewed by Monk (1948). He states that *falciparum infections* present no real therapeutic difficulties, since they can almost entirely be prevented by regular dosage with the main antimalarial drugs, and quite easily eradicated if diagnosis is made early enough and there is no cerebral involvement. Covell *et al.* (1949), however, found that infection with an African strain of

falciparum was not eradicated by treatment with proguanil alone, though combination of this drug with mepacrine, or with quinine, was effective. Similar results were obtained by Chaudhuri (1948) who found that proguanil alone failed to cure infections due to an Indian strain of *P. falciparum*. Hence the treatment of infections with certain strains of this parasite still presents some difficulty. Covell and his co-workers also found that the clinical attack was controlled more rapidly by mepacrine or quinine than by proguanil, and this too may be of importance since the persistence of serious symptoms in falciparum infections may lead

TABLE 5

Showing the drugs effective at various stages of the life cycle of P. vivax *in man.*

Stage	Drugs effective
Exo-erythrocytic	Proguanil Pentaquine Pamaquin
Asexual	Quinine Mepacrine Pamaquin Proguanil Chloroquine Pentaquine
Sexual	Pamaquin

to various complications. The treatment of malignant tertian malaria is reviewed by Covell (1949).

The main problem at present, however, is the complete eradication of *P. vivax infections* and the ideal drug capable of accomplishing this has yet to be discovered. What appears to be quite clear is that no single drug, given alone, is satisfactory for the cure of established *P. vivax* malaria. Perhaps of all the drugs tried singly, pentaquine shows the lowest relapse rate, though comparatively little clinical work has been done with it to be certain, but combinations of drugs have undoubtedly given the best results. This is rather puzzling since some of these effective combinations, e.g. quinine and pamaquin, include one drug

(quinine) which is not supposed to have any action on the exo-erythrocytic stage of the parasite. If the cure of the disease involves the complete eradication of the exo-erythrocytic forms, thus preventing recurrence after cessation of treatment, it is difficult to understand why the action of pamaquin (or other drugs active against these forms) should be enhanced by quinine which has no action on them. It is possible that quinine does in actual fact potentiate the effect of drugs active on the exo-erythrocytic forms. This question requires further investigation.

Of the combinations of drugs tried in the cure of *P. vivax* malaria, the most important are quinine and pamaquin, proguanil and pamaquin, and quinine and pentaquine.

Before the introduction of proguanil and pentaquine, the combination of quinine with pamaquin represented the standard course of treatment. Quinine, 0.65 G., with pamaquin 10 mg. is given eight hourly for 10–14 days, and produces a radical cure in some 80–90 per cent of patients; toxic effects are uncommon and rarely require modification of dosage.

When proguanil (100 mg.) is combined with pamaquin (10 mg.) and given 8 hourly, the results are at least as good as those obtained with the quinine-pamaquin course. With this dosage side effects are almost negligible, and it may well be that this is the optimum combination of those so far investigated.

Preliminary results suggest that quinine 0.65 G. and pentaquine 20 mg. at 8 hourly intervals may be even better, but, according to Monk (1948), the general malaise and anorexia of the great majority of patients receiving this course was in marked contrast to the good health and appetite of those treated with quinine-pamaquin. Good results with such a combination in relapsing cases of vivax malaria have been reported by Straus and Gennis (1948), and also by Coggeshall and Rice (1949) who state that toxic reactions were not sufficient to cause concern, and were largely those commonly associated with the use of quinine.

Proguanil is of outstanding value as a *prophylactic*. In doses of 100 mg. once daily it can produce complete suppression of all manifestations of malaria, although it does not prevent the establishment of vivax infection. This treatment may supersede the use of mepacrine, and possibly also chloroquine, for this purpose, though further investigation of the relative value of these drugs is necessary.

Conclusions

It is obvious that the problem of malarial control is not yet solved, though great advances have been made, and the discovery of exo-erythrocytic forms in the liver of primates should be a great help in the experimental approach to the problem. Proguanil is undoubtedly of great value as a suppressant, and its low toxicity allows it to be taken easily over long periods. Two other new drugs have been investigated and may well replace older compounds. Chloroquine is less toxic and more effective than mepacrine in the treatment of malaria and, in addition, does not stain the skin. If the results with this compound are confirmed it will mean that mepacrine should no longer be used. The case of pentaquine is not, as yet, convincing. It has actions similar to pamaquin and may be less toxic and more effective but this requires further investigation.

No single drug is satisfactory for the complete cure of *P. vivax* malaria. Combinations of quinine, or proguanil, with pamaquin, or pentaquine, are more efficacious than any one drug, and one of these combinations undoubtedly represents the best method at present available for the treatment of *P. vivax* malaria. *P. falciparum* infections are much more easily eradicated and proguanil is probably the best drug for this purpose, though it may have to be combined with pamaquin or quinine in infections with certain strains. There is now no justification for using quinine alone in the treatment of malaria; it is still probably true, however, that this drug acts more rapidly than any other in controlling a particular attack, though it has little or no effect in preventing relapses.

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CHAPTER 9

ANTRYCIDE

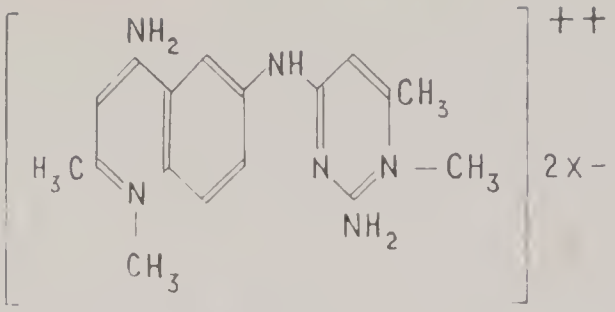
Large areas of good pasture lands in some parts of Africa are unsuitable for cattle and other livestock because of the incidence of trypanosomiasis carried by the tsetse fly.

The disease could be controlled by the eradication of the fly but this is a long and costly process which has, however, already achieved some success. Much can be done to decrease the consequences of the infection by improving the nutrition and water supply of the cattle. Nevertheless a drug which could control the disease would still be of outstanding value, greatly increasing the supply of meat and other animal products.

There are a number of species of trypanosomes and their reaction to drugs varies greatly. *T. congolense* is probably of greatest significance, though the recent work described by Davey suggests that *T. vivax* is also very frequently found in cattle. A great advance in the chemotherapy of *T. congolense* in cattle came with the introduction of phenanthridinium compounds by Browning and his co-workers in 1938; the most important of these, compound 1553, was named dimidium bromide. Antrycide, discovered by Curd and Davey, represents a further striking advance in this field, though it still does not appear to be the complete answer to this chemotherapeutic problem. Much further work remains to be done with it and only a brief discussion is possible at this stage. We are greatly indebted to Dr. Davey for some of the unpublished information and recommendations given below. Incidentally, experimental work with cattle in these regions is not particularly easy, and the investigator has to contend with a number of unusual difficulties, including sometimes the protection of his animals against lions!

Constitution and Pharmacology

Antrycide is the name given to 4-amino-6-(2′-amino-6′-methyl-pyrimidyl-4′-amino) quinaldine-1 : 1′-dimetho salts and the formula is shown on p. 211 (Curd and Davey, 1949).



Antryeide

where x is Cl(antryeide chloride) or CH_3SO_4 (antryeide methylsulphate). These are both white crystalline solids which appear to have equal trypanocidal activity, once they are brought into contact with trypanosomes, but they possess widely different solubilities in water which give them different pharmaeologieal properties. The methyl sulphate is soluble to the extent of about 33 per cent in water, whereas the chloride is soluble only to the extent of 0.12 per cent. After subcutaneous or intramuscular injection the chloride is absorbed slowly, the methyl sulphate

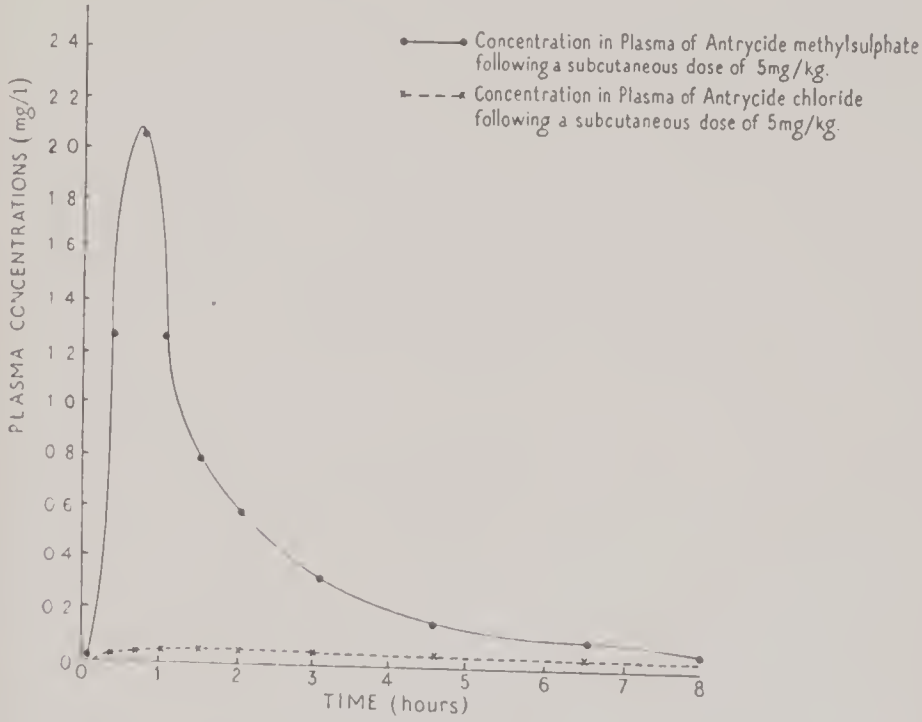


FIG. 22

The concentration of Antryeide in the plasma of calves following subcutaneous injection.

rapidly. Spinks (1949) has developed a fluorimetric method for the determination of antrycide, and has determined the concentration of the drug in the plasma of calves following the subcutaneous administration of comparable doses (5 mg./kg.) of the chloride and methyl sulphate (Fig. 22).

Owing to its low solubility, antrycide chloride, when given by injection, forms a depôt which is slowly absorbed and this salt is therefore suitable for prophylaxis. As will be seen below the duration of prophylaxis in cattle is not as long as might be desirable, and it is quite likely that other preparations of antrycide, giving effective chemotherapeutic concentrations for prophylactic purposes, will be made and investigated.

Trypanocidal Properties

Figures showing the relative curative activities of antrycide and of dimidium bromide in mice infected with various strains of trypanosomes are shown in Table 6, while the prophylactic effect of antrycide is illustrated in Table 7.

TABLE 6

Minimum curative dose (mg./kg.) of antrycide and dimidium bromide in mice infected with various strains of trypanosomes.

Drug	T. rhodesiense	T. equiperdum	T. equinum	T. evansi	T. congolense
Antrycide (S.C.)	12.5–25	0.5	1.0	2.5–5	1–2
Dimidium bromide (I.P.)	>25	>25	>25		2–4

TABLE 7

Prophylactic effect of antrycide in mice against attempted infection with T. congolense. *The figures show the number of mice which resisted infection at various periods after the prophylactic dose of the drug.*

Dose of antrycide chloride (mg./kg.)	Results—weeks after treatment 2	4	6	8	10
25	10/10	8/8	6/6	7/8	1/3
12.5	8/8	8/8	1/6	0/8	
5	3/10	0/8			

Toxicity

The methyl sulphate is more toxic than the chloride when given by subcutaneous injection; for example, mice have been given more than 1 gram/kg. and rabbits more than 200 mg./kg. of the chloride without any marked general toxic effect, whereas much smaller doses of the methyl sulphate produce toxic effects, the M.L.D. in mice being 20–30 mg./kg. subcutaneously, 15–20 mg./kg. intraperitoneally, and 10–15 mg./kg. intravenously. These differences between the two salts are of course due to differences in solubility. From the evidence so far available the therapeutic index of the substance in cattle is not particularly high. The suggested therapeutic dose of the sulphate is 5 mg./kg. but a dose in excess of 10 mg./kg. can be toxic (Wilson, 1949). The toxic symptoms sometimes develop quickly, the animal losing consciousness and dying two to six hours after treatment; in other cases a severe gastro-enteritis develops 48 hours after treatment, and death occurs between the sixth and twelfth days. Further investigation of the question is desirable. Davey recommends that animals should be kept as quiet as possible for 12 hours after treatment, since undue exertion may cause fatalities. Local reactions at the site of injection are not infrequent but are not serious.

Clinical Use

It is much too early to try and sum up the value of antryeide in the prophylaxis and treatment of trypanosomiasis in cattle and other domestic animals, but it is quite clear that one of the major difficulties is the development of resistant strains of the organism. The drug tolerance is retained when the strain is transmitted to clean animals by the subinoculation of blood, and drug tolerant strains of infection are not eradicated by full curative doses of the drug. This makes it particularly important that the drug should not be mishandled, and that it should be given for both prophylactic and therapeutic purposes in doses which will truly kill all organisms in the body and not allow of the development of resistant strains. This is all the more important since there is evidence that organisms resistant to antryeide are resistant also to curative doses of dimidium bromide (Wilson, 1949). This is rather unexpected and further investigation of this question is necessary.

It seems quite clear that in full therapeutic doses (5 mg./kg. of the methyl sulphate subcutaneously) antrycide is curative against primary infections of trypanosomiasis due to *T. congolense*. It has also been used successfully against some strains of *T. vivax* but, according to Davey, more information is required before a sound general picture of the behaviour of *T. vivax* towards antrycide can be formed.

The preliminary results of a large field trial of antrycide started in January, 1949, in the Southern Sudan are interesting (personal communication from Mr. J. T. R. Evans). In this extensive area believed to be free from tsetse flies, 20–30 per cent of the cattle showed symptoms of trypanosomiasis; *T. congolense* was responsible for most of the cases and *T. vivax* for the remainder. The plan was to treat *all* the cattle in an area in order to ensure that the subclinical as well as the clinical cases were inoculated. It was thus hoped to produce an extensive block of cattle that was virtually free from trypanosomiasis.

By the end of June, 1949, 200,000 cattle in an area of about 20,000 square miles had been inoculated with antrycide sulphate given subcutaneously as a 15 per cent solution at an estimated dosage of 5.0 mg./kg. About 10,000 of the cattle, many of them from the worst infected herds, were inspected 3–4 months after treatment and were seen to be in good condition. Blood examinations of representative groups in many herds were made but no trypanosomes were found.

About a quarter of the treated cattle showed copious salivation which lasted for several hours. Two or three animals in a thousand collapsed immediately after inoculation but most of them recovered an hour or two afterwards. Early in the campaign a few young calves collapsed and died within 24 hours and it was then decided not to inoculate any more under the age of six months.

It should be noted that very good results have been obtained with dimidium bromide in the treatment of cattle infected with *T. congolense* in the Southern Sudan (Evans, 1948). Over 350,000 cattle were treated in one area and the mortality from trypanosomiasis reduced from alarming proportions to negligible numbers. Symptoms of photosensitization appeared in some of the treated cattle, particularly among those which were short of food and water after administration of the drug.

The prophylactic effect of antrycide methylsulphate is inferior to

that of the chloride, but since it should be assumed that under general field conditions some at least of the cattle being injected for prophylactic purposes may be infected with trypanosomes, a mixture of the two salts providing both a curative and a prophylactic action should be used in prophylaxis. It appears that protection against *T. congolense* lasts for 4–6 months, but against other species such as *T. vivax* the period of protection may be much shorter in a proportion of animals. The period that may be allowed to elapse between treatments will vary in different areas, but in most areas it seems the treatment ought to be given about four times a year. This is not usually feasible in practice under the conditions prevailing in infected areas, but it is possible that the production of a form of the drug which produces an effective depôt for longer periods may lead to a solution of this problem.

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CHAPTER 10

CHEMOTHERAPY OF MALIGNANT DISEASE

The rapid progress in the chemotherapy of bacterial infections, which at one time seemed impossible of achievement, has encouraged the hope that similar methods might be of value in dealing with the growth of malignant cells. It is generally accepted that bacteria can be specifically influenced by certain substances in the body, because their metabolism is different from that of the host organism and is therefore decisively affected by substances which have little or no effect on the metabolism of the host organism. It should, in the same way, be possible to influence specifically the metabolism of malignant cells which must surely differ significantly from that of normal cells: it is, at least, difficult to explain otherwise the relative independence from their environment which malignant cells show in their growth processes. At the same time it must be admitted that little progress has been made in elucidating in what way the metabolism of malignant cells differs from that of their normal neighbours, though such knowledge should form the basis of a rational attack on the problem. Greenstein (1947) discusses differences between enzyme systems of normal and malignant tissues. Such advances in the chemotherapy of cancer as have been made have resulted essentially from an approach not based on knowledge of metabolism, and they have not, so far, resulted in the discovery of any substance which will cure any form of malignant disease. Nevertheless they are interesting and some of them may pave the way for more important advances of theoretical or practical interest. It must not be forgotten that the discovery of the sulphonamides, based as it was on an essentially empirical scheme of investigation, preceded the formulation of the theory which is believed to account for the action of these substances.

Selective Actions. One point of interest is that the drugs so far discovered which have an effect on tumour growth do not affect

all forms of tumours equally. On the contrary, they appear to exert a rather selective action on a particular type of tumour. Thus œstrogens inhibit the growth of cancer of the prostate, nitrogen mustard acts in Hodgkin's disease, urethane is effective in leukæmia, etc. All these substances, therefore, do not act on a metabolic activity, or enzyme system, peculiar to malignant cells, but rather each of them appears to affect a metabolic function of a particular type of abnormal growth. It is possible that ultimately a substance, or substances, will be found which acts more or less equally on all types of tumours. In the meantime, however, it is important that drugs which are being considered as potential therapeutic agents for cancer should be tested against a wide range of tumours. Boyland (1948), who emphasizes this point, suggests that such substances should be investigated not only against a well established transplantable tumour, such as the Walker rat carcinoma, but also against series of tumours in mice, including a mammary carcinoma, a sarcoma, a hepatoma, a prostatic carcinoma, a lung tumour, a lymphosarcoma, a gastric carcinoma, and a leukæmia. Such widespread testing of their activity would be more likely to reveal an action against a particular type of tumour in the same way as the testing of antibiotics against a range of micro-organisms has led to the discovery of substances which act specifically on certain types of bacteria.

Types of Substances Used. It is interesting that the main substances so far found of some value in the treatment of malignant disease can be divided into two groups, namely (1) *those that affect the activity of the nucleus*, i.e. nitrogen mustard, radiophosphorus, urethane, and (2) *those that prevent the production, or antagonize the action, of a specific hormonal growth stimulus*, i.e. oestrogens and androgens.

The substances in the first group also affect growth of normal cells, but their action on certain malignant cells is more marked. This applies too, to radiations, viz. X-rays and radium, which probably also have a rather selective action on the nucleus of some forms of malignant cells, and are of value in the treatment of cancer. Further investigations into the activity of the cell nucleus, and of substances which modify this activity, may well lead to progress of value in the control of malignant cells.

The hormonal substances, œstrogens and androgens, have proved of value only in malignant disease involving sex organs,

namely the prostate and the breast. Cancer of these organs is peculiar in that its growth is stimulated by steroid hormones normally produced in the body ; the inhibition of this stimulus by the appropriate substance will therefore decrease or even temporarily arrest the growth of the malignant process. It is not known how the steroid hormones stimulate the growth of the sex organs but it is quite possible that their action is exerted on the cell nucleus ; if this is so, then the appropriate inhibitors would also presumably act on the nucleus, thus acting on the same site in the cell as the first group of substances. An additional point deserves consideration. It is probable that the growth of cancer cells in sites other than the sex organs is also under the control of one or more substances. Investigations of such possible growth factors, and of suitable antagonists, is one method by which chemotherapeutic substances active against cancer might be discovered. In this connection the work which is at present in progress on the effect of folic acid and its antagonists on malignant growth is of special interest. This work is still in its early stages, but it has already been shown that temporary remission in acute leukæmia can be produced by the administration of several folic acid antagonists (Farber, 1949 ; Dameshek, 1949). The use of folic acid antagonists in neoplastic disease is reviewed by Thiersch and Philips (1949). Several 4-amino-folic acids have been used, viz. *Aminopterin* (0.5–1 mg. per day), *Amethopterin* (3–5 mg. per day) and *Amino-an-fol* (25–50 mg. per day). Folic acid antagonists are also discussed in Chap. 15, as are the interesting experimental findings with a purine antagonist.

Œstrogens and Androgens

These have been used in the treatment of certain forms of cancer involving the sex organs. The most striking effects have been seen with œstrogens in cancer of the prostate, though even here the condition is only temporarily controlled. Œstrogens, as well as androgens, have also been used in cancer of the breast, but the effects are not marked and do not last very long.

Cancer of the Prostate

Huggins (1942) showed that in this condition castration is frequently followed by a sustained inhibition of the disease. This is most striking in cases where the neoplasm is an adeno-carcinoma,

while in undifferentiated carcinomas the improvement is slight or nil. The growth of malignant prostatic tissue, both primary and secondary, is stimulated by androgens and it is to be presumed that this stimulant action is quite marked in the prostatic adenocarcinoma and slight or absent in the undifferentiated type of tumour. A reduction in the effects of androgenic stimulation can also be produced by the administration of œstrogens, which probably produce this effect by at least two mechanisms, viz. :—

(1) By an inhibition of the pituitary followed by a cessation of testicular androgenic secretion, which is controlled by the pituitary. This is thus equivalent to castration as far as the internal secretory activity of the testes is concerned.

(2) By an inhibition of the action of testosterone on the prostate. It is well known that œstrogens can inhibit the actions of androgens on a number of sex organs. It is possible, in addition, that œstrogens may have a direct action on the growth of malignant tissue, though this effect is probably slight. Horning (1949) has produced experimental prostatic tumours in mice, by implanting subcutaneous grafts of prostatic epithelium impregnated with methyl cholanthrene, and studied the effect of stilbœstrol on their growth.

The results of Huggins led to an investigation of the action of œstrogens in cancer of the prostate, with striking results (Herrold, 1941 ; Heckel, 1944). The administration of these substances causes a prompt relief of pain and urinary symptoms ; this is associated with a regression of the primary and metastatic tumours. Degenerative changes may be produced in the tumour tissue, as has been demonstrated by histological examination (Heckel and Kretschmer, 1942). The effects in the tumour can be correlated with the changes which occur in the clinical condition (Fergusson and Pagel, 1945).

Stilbœstrol is the œstrogen which has been mainly used in the treatment of cancer of the prostate, though there is no evidence that any particular œstrogen has a specific effect. The doses used are rather high, e.g. 3 mg. or more of stilbœstrol per day, given orally. Good results have also been reported with the chloro- and bromo compounds of triphenyl ethylene (Berger and Buu-Hoï, 1947). Cases resistant to stilbœstrol have been improved by the administration of triphenyl bromo ethylene in doses of 20 mg. per day. Triphenyl ethylene compounds are eliminated from the

body much more slowly than stilbœstrol and hence a constantly high and effective concentration can more easily be maintained with such compounds.

The prolonged administration of these large doses of œstrogen produce unpleasant side effects which are, of course, of minor importance in such a serious condition. The breasts become enlarged and often painful; atrophic changes occur in the testes, with arrest of spermatogenesis; there may also be œdema which affects the lower extremities and may involve the scrotum and penis (Wattenberg and Rose, 1945). Occasionally vertigo occurs, and this may be the precursor of a cerebral catastrophe (Fergusson, 1946).

Acid Phosphatase. Androgens stimulate the secretion, by the prostate, of an enzyme, acid phosphatase, which catalyses the splitting of phosphoric acid from certain phosphoric esters, and produces its optimum effect in an acid medium of pH 5.0. The enzyme is probably secreted by the acinar epithelium, and high concentrations of it are present in the prostate of adults, but not of children (Gomori, 1941). Under normal conditions the amount of acid phosphatase in the plasma is low. It is markedly increased in cancer of the prostate with secondaries, but not if secondaries are absent (Gutman, 1942). The amount of acid phosphatase in the plasma can therefore be used as a diagnostic procedure, and also as an index of the response to treatment, by castration or by the administration of œstrogen (Dean *et al.*, 1944; Watkinson *et al.*, 1944).

The polarographic method (see p. 346) has also been used as an empirical aid in the diagnosis of cancer of the prostate, and in assessing the value of treatment. Typical curves (polarograms) are obtained with the serum of normal subjects, and abnormal curves are frequently seen in patients suffering from cancer (and certain other conditions). The clinical improvement observed in prostatic cancer with the administration of œstrogen is accompanied by a return of the polarogram to the normal. It may well be that this method will prove of value as an objective measure of the results of chemotherapy in other forms of cancer (Robinson, 1949).

The effect of stilbœstrol does not continue indefinitely. Patients who show a satisfactory initial response, as judged by clinical, biochemical and histological criteria, often relapse quite suddenly,

as if the sensitivity to œstrogen was abruptly lost (Fergusson, 1946). The reason for this is unknown.

Cancer of the Breast

Both *œstrogens* and *testosterone propionate* have been used in the treatment of this condition (Council on Pharmacy and Chemistry, 1947 and 1949; Adair *et al.*, 1949). It is emphasized that hormone therapy should be reserved, in everyday clinical practice, for patients in whom the possibilities of adequate surgical treatment and careful roentgen irradiation have first been fully utilized and have given clear evidence of being no longer of benefit. Testosterone may be of value at any age in patients with metastatic lesions. The favourable effects of œstrogens, on the other hand, are confined to soft tissue manifestations in older women, and œstrogens should not be given to any patient who is still menstruating or who has menstruated within a period of five years, as this may actually accelerate the rate of growth of the carcinoma. The most suitable patients for œstrogen therapy are about sixty years of age or over. Any effect produced with androgen or œstrogen is always temporary and ultimately the malignant process pursues its remorseless course. The mode of action of the drugs is by no means clear. There is evidence that ovariectomy can be of value in cancer of the breast, presumably because the œstrogen secreted by the gonads stimulates the growth of the malignant as well as the normal mammary tissue, and this stimulus is no longer present when the ovaries are removed. Testosterone will have a similar effect, by antagonizing the action of œstrogens on the growth of the malignant tissue. In addition, testosterone will also have an indirect effect on bony metastases, by increasing the sclerosis in the local bone lesion; this is due to the laying down of osteoblasts (Adair, 1948). Experimental evidence on the action of androgens on tumours is reviewed by Huggins (1947). The mode of action of œstrogens is even more obscure. The effect is possibly exerted directly on the tumour, since the administration of œstrogen will inhibit the growth of a transplanted sarcoma in rats (Robson and Scott, unpublished observations), a finding which led to the investigation of œstrogens in various types of cancer by Haddow and his co-workers. The mode of action of œstrogens requires, and deserves, further study.

Adair (1948) reviews the literature on the use of androgen in

cancer of the breast and also discusses his own results. The dosage needed is high and Adair recommends the intramuscular administration of 100 mg. of testosterone propionate three times a week for ten weeks, followed by a daily maintenance dose of 40–60 mg. of methyltestosterone given orally. The treatment is of value only in certain cases, but the effect may be marked, especially in cases with bony metastases. There is then relief of pain, gain in weight, ability to sleep without narcotics and a feeling of well being, in addition to an effect on the growth of the tumour tissues, which is occasionally striking.

These large doses of androgen produce masculinizing effects, including hirsutism, sometimes accompanied by acne, and deepening of the voice associated with huskiness. An increase in sexual desire takes place except in women of about sixty or older, and this effect is sometimes marked enough to require sedation and local applications to the area of the clitoris.

Three synthetic œstrogens have been used in cancer of the breast, namely triphenyl chloro ethylene, triphenyl methyl ethylene, and stilbœstrol (Haddow *et al.*, 1944). These drugs are given orally, the daily doses being 10–15 mg. for stilbœstrol and 3–6 grams for the triphenyl ethylene compounds. In some cases this treatment produces a significant, though temporary, retardation, or even partial regression of the growth of the primary tumour, soft tissue recurrances, lymph nodes and pulmonary metastases. The degree of retardation is, however, less than can be expected from local application of X-rays. There is no evidence that œstrogens will prevent the development of metastases. In favourable cases there is a definite improvement in the general physical condition of the patient.

The Nitrogen Mustards (β-chloroethylamines)

These substances (see formulæ on p. 234) were extensively investigated during the war and the fundamental results then obtained led to their introduction into medicine (see review by Gilman and Philips, 1946). It is clearly established that mustard gas and the nitrogen mustards have a remarkable effect on growing tissue. They act on the bone marrow producing leucopenia, thrombocytopenia and anæmia; on the intestine inhibiting growth and leading to diarrhœa and vomiting; and on the lymphoid tissue causing lymphatic atrophy. Such effects can be

(*a*) (*b*)

FIG. 23

(*a*) Cell of Walker rat carcinoma in anaphase of mitosis showing the equal distribution of the daughter chromosomes to the opposite poles.

(*b*) Cell of Walker rat carcinoma in anaphase of mitosis 48 hrs. after injection with nitrogen mustard. Several of the chromosomes are fragmented, the fragments are left scattered in the cytoplasm and will be lost from the nuclei. A nucleus deficient in chromosomes will cause death of the cell. Magnification ca. 6,600.

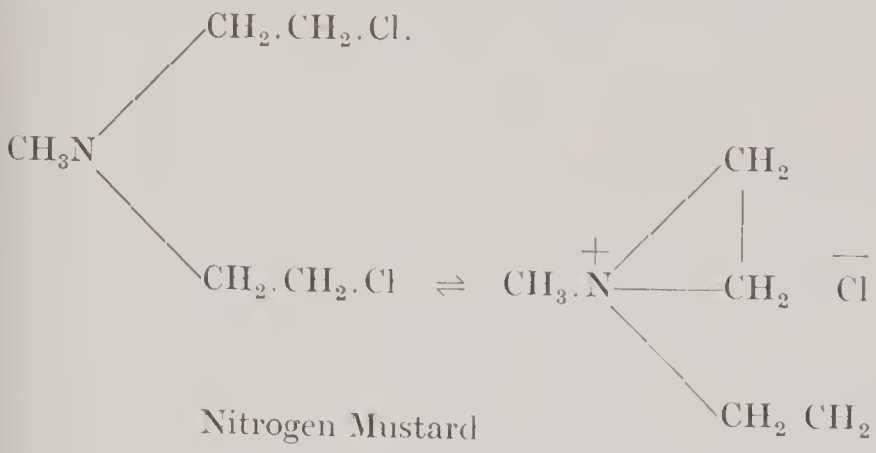
(Very kindly supplied by Dr. P. C. Koller.)

[*To face page* 222.

demonstrated experimentally. For example, if the vaginal epithelium is treated with one of these substances, mitosis is inhibited for a long period during which time the epithelium will not respond by growth to the administration of œstrogen. This action can also be seen in lower forms of life. Thus exposure of yeast cultures to mustard gas produces an immediate reduction in growth rate which persists through several generations. There is much evidence that these effects are exerted on the cell nucleus, and the mutagenic action of these substances will be discussed in Chap. 11.

There has been much speculation as to why these substances are so active on the nuclei of growing cells. It has been attributed to their ability to form cyclic onium cations, which are extremely reactive with various groups present in tissues, e.g. amino, imino, imidazol, sulphydryl, etc.

The cyclic reaction for nitrogen mustard is as follows :—



Nitrogen Mustard

Cyclic compound

If this is indeed the mechanism of their action, the wonder is not that effects are produced but that, in low concentrations, such comparatively specific effects can be obtained on the nuclear material. In part this may be due, as Boyland (1946) has suggested, to the fact that they are lipoid soluble substances of low molecular weight, which penetrate readily into the cell where they combine with essential cell constituents, which prevents their rapid removal.

These substances are capable of inactivating enzymes and Dixon and his co-workers have obtained much evidence that their

vesicant action, at least, is closely connected with their capacity to inactivate, almost specifically in low concentrations, certain enzymes (phosphokinases) which are concerned with the transfer of phosphate to and from compounds of adenylic acid (Dixon and Needham, 1946). It is likely, however, that effects on the nucleus can be produced by concentrations of these vesicants lower than are necessary to affect the phosphokinases. It is also possible that other enzymes, e.g. peptidase, are involved in the nuclear actions.

Clinical Results. The action of the chloroethylamines on growing tissues led to the investigation of their value in malignant disease. This had previously been attempted by Berenblum (1931) with mustard gas, but the results he obtained experimentally in mice were not satisfactory. Gilman in America (1946) and Wilkinson in Britain (1947), and their collaborators, initiated clinical investigations with nitrogen mustards, which have the advantage that their hydrochlorides are solids freely soluble in water. Most of the work has been done with methyl-*bis* (β-chloroethyl) amine hydrochloride. Experimental investigations with other similar compounds have not so far led to the discovery of any more effective substances (Shapiro *et al.*, 1949). The effects have been investigated in a number of neoplastic and proliferative diseases of the hæmopoietic and lymphatic tissues, viz. Hodgkin's disease, leukæmia, lymphosarcoma, polycythæmia vera, etc. (Wilkinson and Fletcher, 1947; Wintrobe *et al.*, 1947; ApThomas and Cullumbine, 1947; Zanes *et al.*, 1948). Interesting experimental results in animals have also been obtained by Karnofsky *et al.* (1947) and Boyland (1948). The most satisfactory clinical results have been obtained in *Hodgkin's disease* in which the treatment usually produces a remission lasting several months: there is reduction of the gland masses, regression of the enlarged liver and spleen, and a great improvement in the general condition. Favourable effects have also been seen in chronic myeloid leukæmia and in polycythæmia vera. Cancer of the lung too, occasionally responds to this treatment (Boyland *et al.*, 1948; Karnofsky *et al.*, 1948; Brown and Davis, 1949). The effects are in all cases temporary and the treatment must be repeated every few weeks or months as in the case with X-rays. Ultimately the disease runs its usual course. The histological changes found in patients who were treated with nitrogen mustard are described by Spitz (1948).

Nitrogen mustard is given in a dose of 0.1 mg. per kg. of body weight, repeated on consecutive or alternate days until 3–6 doses have been given. It is a highly toxic substance which has to be used with great care, and given truly intravenously, as the introduction of the slightest amount of nitrogen mustard into the tissues outside the vein will cause a severe local reaction. Nausea and vomiting usually come on within a few hours, and anorexia and headache are very common. Leucopenia and some anæmia are produced, but these effects are temporary and after about five weeks hæmopoiesis is back to normal. With overdosage, however, serious destruction of blood forming and lymphatic tissue may occur leading to aplastic anæmia. At present the use of nitrogen mustard is likely to be restricted to Hodgkin's disease. Its effect is similar to that of X-rays which, according to some investigators, is still the better method of treatment with fewer toxic effects. Nitrogen mustard can, however, still be of value in cases where radiotherapy has been abandoned. According to Dameshek *et al.* (1949) a course of nitrogen mustard may restore the radiosensitivity of the malignant tissue.

Urethane

This substance has long been known as a mild hypnotic and in 1910 Warburg showed that it inhibited cell division and nuclear division of the eggs of sea urchins. Dustin (1947) discusses the effects of urethanes and related substances on cellular division.

Haddow and Sexton (1946) studied the effect of phenyl urethane, and then of urethane, on the growth both of spontaneous and of transplantable cancer in mice and observed an inhibitory action. Urethane had the more marked effect on the transplantable tumour and this was accompanied by a profound modification in its histological appearance, the characteristic cellular structure giving place to a more fibrous structure with an abundant stroma. Lushbaugh *et al.* (1948) studied the effects of urethane on the healing of experimental wounds in rats, and came to the conclusion that the chief mechanism by which this substance retards neoplastic growth is probably by producing an inhibition of capillary proliferation. An inhibitory effect of urethane on lymphatic leukæmia in rats has been demonstrated by Murphy and Sturm (1947). The mode of action of urethane is not really known. One interesting suggestion made by Todd is that it competes with some

natural amine in the synthesis of nucleotides, an effect which might well account for its actions on the nucleus (see Haddow, 1948).

It is of interest that urethane can also cause the appearance of certain types of tumours. This was first observed by Nettleship and Henshaw (1943), who found that the administration of minimal anæsthetizing doses to mice of the C_3H strain increased the incidence of lung adenomata from 5 per cent to more than 75 per cent. Jaffé (1947) observed the same type of effect in rats, in which animals spontaneous lung tumours occur less frequently. All these tumours are benign, but Noble and Miller (1948) have apparently induced malignant tumours in mice by the administration of urethane in a 5 per cent solution of zinc acetate.

Clinical Results. When urethane was administered to patients suffering from cancer the results were negative. It was observed, however, that it produced a fall in the leucocyte count and this led to its trial in *leukæmia* and other lymphadenopathies. It was found that urethane produces remarkable effects in leukæmia (Paterson *et al.*, 1946). In the most favourable cases the leucocyte count falls to within normal limits, there is a diminution in the size of the spleen and enlarged lymph glands, and a rise in the hæmoglobin level. These findings have been confirmed by a number of observers (see *Lancet*, 1948; Hirschboeck *et al.*, 1948; Creskoff *et al.*, 1948). It is clear that the drug is most effective in *chronic myeloid leukæmia* and useless in the acute leukæmias.

Favourable results have also been reported in patients with *multiple myeloma*. Fever, skeletal pain and acute symptoms subsided after two to four weeks of therapy. The blood condition was greatly improved. The serum protein abnormalities characteristic of the disease became less pronounced or disappeared as did the albuminuria and Bence-Jones proteinuria (Loge and Rundles, 1949). The long term results of urethane therapy, however, still remain to be assessed.

A great advantage of urethane is that it can be given orally and is easily obtainable. It has also been given intramuscularly and intravenously. The dose is about 1–2 grams three times a day. As can be seen in Fig. 24 there is a definite latent period before the effect of the drug on the blood picture becomes manifest. Nausea and vomiting are produced quite frequently with the larger doses and are greatly diminished when the drug is given by injection.

There is also some sedative action which is usually an advantage. The blood picture should constantly be watched as agranulocytosis can, very occasionally, occur. The comparative values of urethane, radiation and nitrogen mustard in the treatment of leukæmia remain to be determined. Some aspects of this are discussed by Piney (1948).

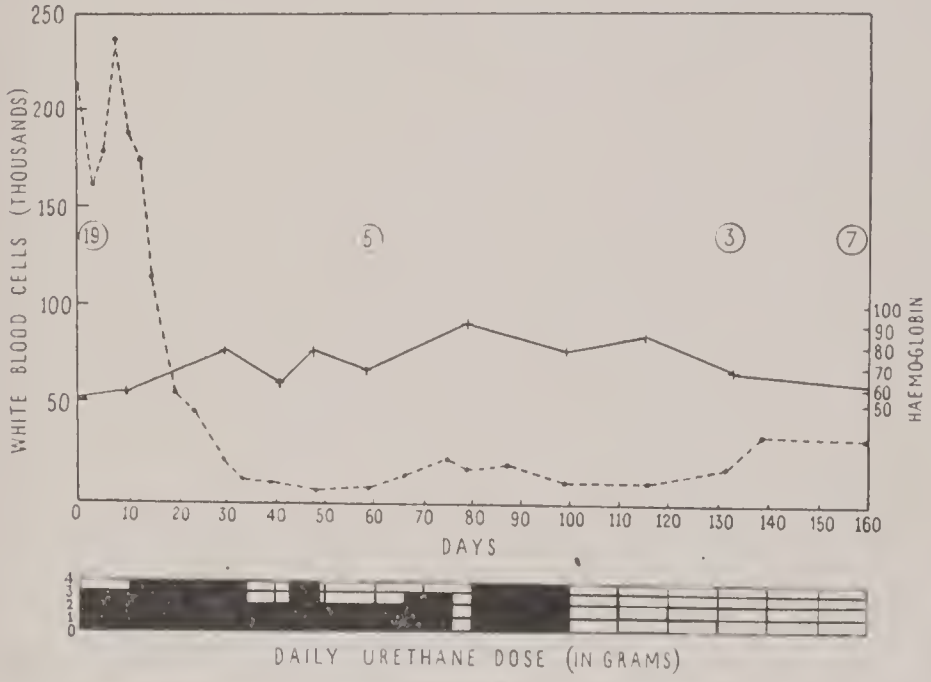


FIG. 24

Showing the effects of urethane treatment in a case of chronic myelogenous leukæmia.

. . . . . White cell count.
Hæmoglobin.
o Size of spleen in cm.

(After Creskoff *et al.*, 1948.)

Radio Phosphorus

This isotope of phosphorus has an atomic weight of 32 and emits only beta radiation. It has a half life of 14.3 days. It has been used, in the form of inorganic phosphate, in a number of conditions involving the hæmopoietic and lymphatic system, as a method of applying radiation to the affected areas. X-rays have been used for these conditions for many years and the radiation is then applied over the whole body. Radio phosphorus could only

be more effective than X-rays if it were selectively picked up and concentrated by the malignant tissue. It would be expected that rapidly dividing tissues would take up phosphorus more quickly than others. There is some support for this in the investigations of Erf *et al.* (1941), who found that leukæmic cells in the blood retain radio phosphorus in greater concentrations than normal white blood cells. The differences are, however, not very marked and it is possible, as Rhoads has pointed out, that more selective effects on malignant cells might be produced by incorporating the radioactive substance in an organic compound, and this possibility is at present being studied.

Absorption and Excretion. Erf *et al.* (1941) have studied the rate of absorption and excretion of radio phosphorus. When given orally, more is excreted in the fæces than in the urine. In the first six days following its administration about one quarter to one half of the dose given could be recovered in the excreta. The maximum plasma concentration was seen before the second hour after administration; after 24 hours lower levels were observed which gradually tapered off to near the zero level on the eighth day. When the drug is given intravenously much more is, as would be expected, excreted in the urine than in the fæces, but the total amount excreted in the first six days following the administration was about the same as when the drug was given orally. The maximum plasma level was higher than with oral dosage, but fell rapidly. Much higher concentrations were, however, found in the white cells, especially in leukæmic patients, and these high levels were maintained for over a week. With both methods of administration normal people excreted radio phosphorus much more rapidly than did patients suffering from leukæmia during the first 48 hours after administration. After that interval, the rates of excretion were similar in both groups. Erf (1942) studied the distribution of radio phosphorus in patients who died from leukæmia; his findings show that radio phosphorus concentrates in greatest quantities in those tissues which are most frequently infiltrated with leukæmic cells.

Clinical Results. The best results with radio phosphorus have been obtained in *polycythæmia vera* and it is probably the best therapeutic agent at present available for this condition. P_{32} has also been used in chronic leukæmia, particularly in patients intolerant of or resistant to roentgen radiation. It is of no value in

acute leukæmia, Hodgkin's disease, or in cancer of various organs (Doan *et al.*, 1947 ; Mitchell, 1947).

There are great variations in the susceptibility to internal radiation of different individuals and tissues, and the dosage must, as Doan *et al.* (1947) have emphasized, be carefully adjusted to each patient's needs. According to these investigators radio

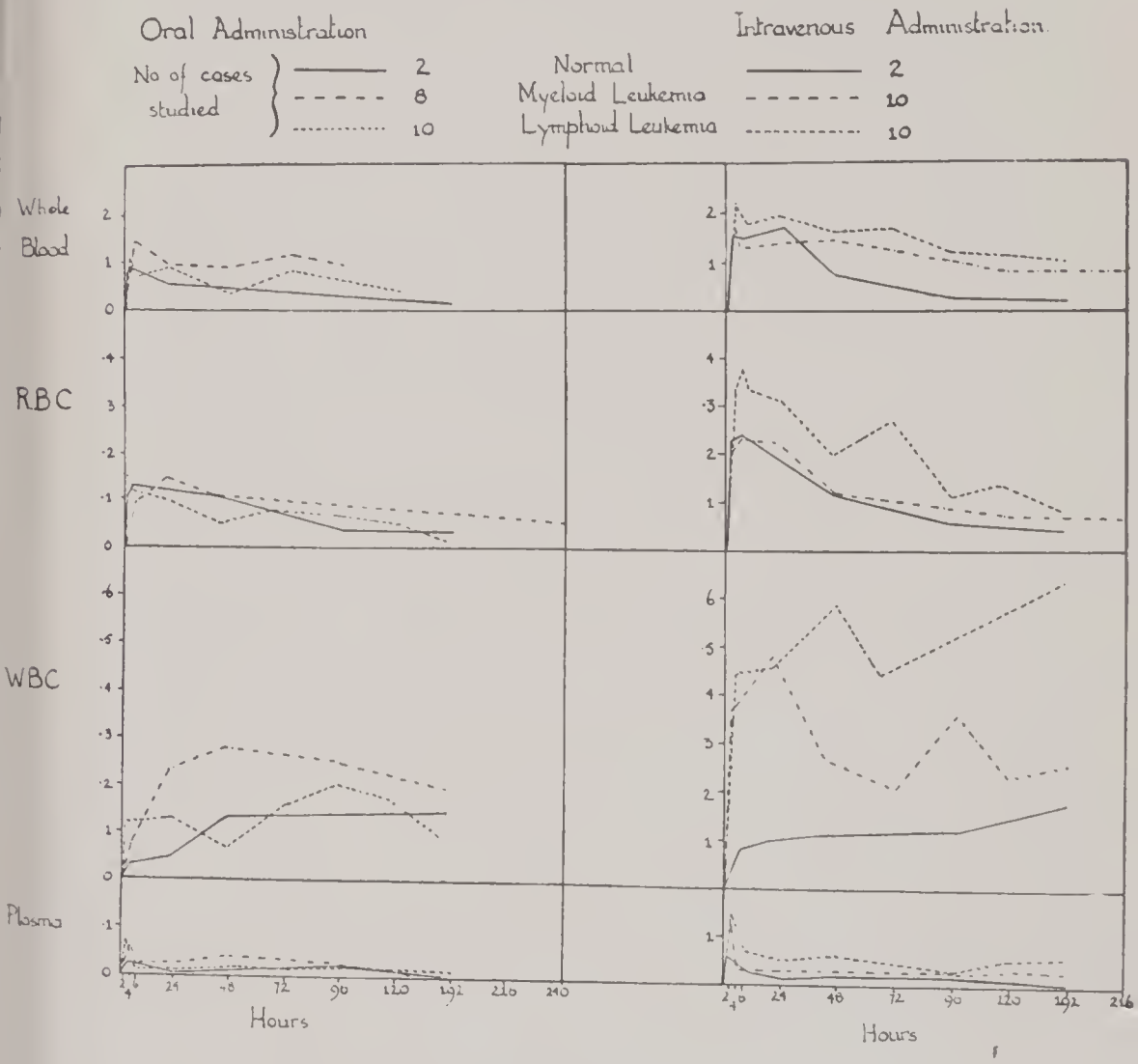


FIG. 25

Showing the distribution of radio phosphorus (P_{32}) in blood fractions of normal subjects and of patients with leukæmia. Note the relatively high concentrations in the white cells in patients with leukæmia, especially when given intravenously.

(After Erf *et al.*, 1941.)

phosphorus should be given in small, well spaced doses, and a careful examination of the blood and bone marrow should be performed frequently, to ensure the integrity of normal hæmopoiesis. The material can be given orally, but it is considered more satisfactory to inject intravenously, and this is certainly in agreement with the findings on the uptake of radio phosphorus by the white cells (Erf *et al.*, 1941). The dose usually given is of the order of 0.5–2 millicuries and this may be repeated several times over a period of months. More frequent doses of 1–2 millicuries have been used by Lawrence and his co-workers (Lawrence *et al.*, 1948) in the treatment of chronic myelogenous leukæmia.

Other radioactive isotopes, e.g. radio sodium (Evans *et al.*, 1948), have been used for the treatment of blood conditions, but these substances do not appear to have any advantages over radio phosphorus.

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CHAPTER 11

MUTAGENIC SUBSTANCES

It is well known that the characteristics of individuals and cells are transmitted by genes. These genes, it would appear, are small particles forming part of the chromosomes in the nucleus of the cells. Each chromosome contains a large number of genes and each gene is located at a definite part of the chromosome. When the cell divides the chromosomes, and hence all genes, are duplicated. The genes are stable and go on reproducing their properties indefinitely.

Occasionally the gene may undergo a change in properties and then it becomes responsible for the control of a new characteristic in the cell or individual. Under such conditions a chemical change presumably occurs in the gene and this is permanent, so that the new gene is as stable as the original one and goes on reproducing replicas of its mutated self, thus initiating a new hereditary line. It seems likely that this mechanism is responsible for evolution and that without mutations life would never have proceeded beyond its very first elementary beginnings.

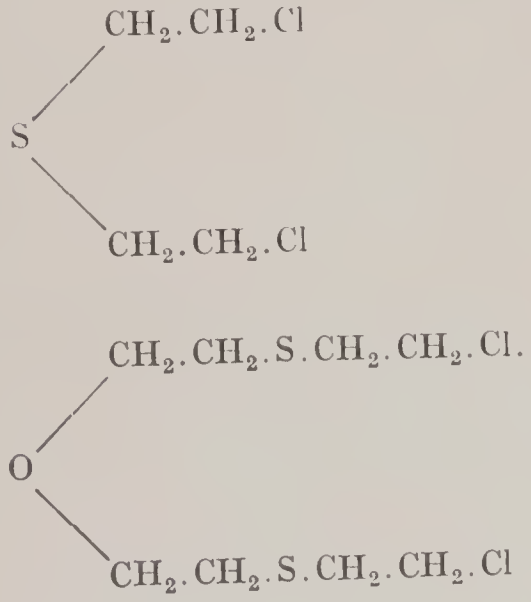
In 1927 Muller found that mutations could be induced in the fruit fly, Drosophila melanogaster, by subjecting it to the action of X-rays, and later work has shown that various other types of radiations, e.g. gamma-rays, ultraviolet-rays, and neutrons can produce the same effect. The effects produced by radiations on the chromosomes are, on the whole, destructive and non-discriminating, so that no specific actions result. Genes are affected at random and it is not possible to produce an effect on a particular gene or group of genes.

Following this work with radiations, attempts were made to produce mutations by means of chemical substances, in the hope that the effect would be more specific and that ultimately it might be possible to produce selective mutations and even to control the process of mutations, and therefore evolution, in definite directions. The early work was essentially negative, but in 1940–41, in an attempt to discover an explanation for the curious actions

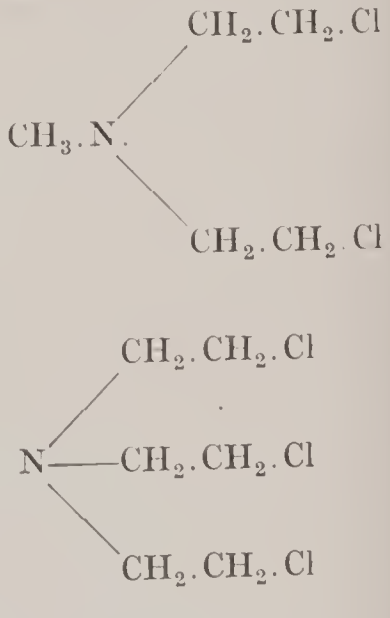
of mustard gas on tissues, it was found that this substance could inhibit mitosis in various tissues and produce mutations in Drosophila (Auerbach and Robson, 1942 ; 1947). Gross effects on chromosomes, resulting in breakage, were also obtained and detected by means of genetic methods, and this led to the investigation of the actions of mustard gas on an organism (tradescantia) in which the actions on chromosomes could easily be observed cytologically. With large doses of mustard gas the effects on the chromosomes were found to be grossly destructive and finally led to the death of the cell, but with smaller doses effects were less destructive and resulted in various kinds of rearrangements of the chromosomes (e.g. inversions, deletions and translocations), some of which were not lethal to the cell (Koller *et al.*, 1943 ; Darlington and Koller, 1947). Changes in the nuclei of mouse skin subjected to the action of mustard gas were observed by Fell and Allsopp (1943).

It was then found that substances related to mustard gas, of which the formulæ are given below, could produce similar effects.

Mutagenic Substances.



Sulphur mustards.



Nitrogen mustards.

$CH_2 = CH.CH_2.NCS.$

Mustard Oil (Allyl iso-thiocyanate).

The mutagenic action of mustard oil (Auerbach and Robson, 1944) is of particular interest, since this substance occurs naturally in certain plants and this raises the possibility that spontaneous mutations may be of chemical origin. Mutagenic effects on Drosophila, following exposure to formaldehyde, have since been described by Rapoport (1946) and by Kaplan (1948).

Loveless and Revell (1949) have found that a number of di-epoxides, including di(2:3-epoxypropyl) ether, have cytological effects similar to those of nitrogen mustard; these investigators give an interesting discussion on the mode of action of "mitotic poisons."

The effects of all these substances are generally very similar to those of radiations and the production of specific mutations has not so far been accomplished.

An interesting development of this work is the application of nitrogen mustard in the treatment of malignant conditions and especially Hodgkin's disease, which is discussed in Chap. 10.

The mutagenic nature of these substances has been confirmed by a number of other observers on different types of organisms, e.g. on neurospora by Horowitz *et al.* (1946), on Aspergillus (Hockenhull, 1948), and on Penicillium notatum (Stahmann and Stauffer, 1947).

Mutagenic effects have also been reported with other substances by Ochlkers (1943) and by Hadorn and Niggli (1946). The latter observers used an interesting new technique which should be capable of wider application. The gonads of full grown larvæ are actually dissected out, exposed to a solution of the chemical substance, and re-implanted into a host larva. With this method a high percentage of mutations was apparently obtained with as simple a substance as phenol. The work requires confirmation.

It has long been believed that cancer arises as a result of a mutation in a somatic cell, which makes it more independent of its environment and enables it to go on dividing to the detriment of the organism. Some investigators have tried to demonstrate that carcinogenic substances were mutagenic, e.g. by feeding Drosophila flies on a medium containing carcinogenic substances or even by injecting colloidal solutions of the carcinogenic substance into the flies, but these attempts were unsuccessful. Demerec (1947) used the technique of exposure devised by Auerbach and Robson and was able to produce mutagenic effects with a number of

carcinogenic substances. His preliminary results suggest that there is a definite correlation between the carcinogenic and mutagenic actions of a variety of substances, and a similar relationship has been found by Latarjet *et al.* (1949), in investigations on the mutagenic action of carcinogens on E. Coli. The question is also discussed by Strong (1949).

It is not known how chemical substances produce their mutagenic action, or whether the mechanism is essentially different from that involved in the effect of radiations. One possibility is that there is a permanent change in the constitution of the nucleoprotein which, in all probability, forms part of the gene. With this possibility in mind the effect of the sulphur and nitrogen mustards on the nucleoproteins of plant viruses was investigated by Cockerham *et al.* (1944) and at Robson's suggestion by Bawden and Pirie (1944). It was found that these substances destroy the infectivity of the viruses without producing any definite chemical change, and notably without separating the nucleic acid from the protein component. No mutagenic effects on the viruses were observed. It is interesting that radiation too can inactivate viruses without producing any change in their chemical composition (Bawden and Pirie, 1938). Gullant and his co-workers (1948) found that mustard gas reacts with nucleic acid to form a compound which contains sulphur but no chloride, and suggest that this may provide a chemical basis for the effects of mustard gas on the nucleus. Chemical mutagenesis is reviewed in detail by Auerbach (1949).

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CHAPTER 12

ANALGESIC DRUGS

In this chapter some aspects of the actions of drugs used in the relief of pain will be discussed. Drugs can relieve pain in a number of ways. For example, spasmolytic drugs can give relief by producing relaxation of certain types of smooth muscle spasm; glyceryl trinitrate acts thus to prevent or abolish anginal pain, in which its vasodilator effect is responsible for an improved blood supply to the relatively ischæmic heart muscle; the relief of migraine headache by intravenous injection of ergotamine tartrate is probably due to a reduction in amplitude of pulsation of the temporal and other arteries of the scalp. Pain due to bacterial inflammation may of course be relieved by appropriate chemotherapy. Again, pain may be relieved by interruption of nerve pathways, as with injection of local anæsthetics for a temporary action, or with ethyl alcohol for a long-lasting effect.

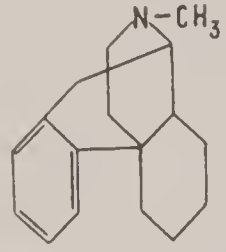
It is, however, with drugs that act upon the central nervous system that this chapter is chiefly concerned, and of these, morphine and its derivatives, and the newer analgesics such as pethidine and amidone, will be discussed at some length. It is not proposed to make more than passing references to what Fourneau (1938) has termed the "antalgics," namely aspirin, phenacetin, antipyrine and amidopyrine.

Another interesting group of compounds are the tetrahydrocannabinols which are euphorigenic in action, and have also been shown to be analgesic in rats (Davies *et al.*, 1946). It would be important to know whether these hemp drugs could, by contributing a euphoriant component, enhance the efficacy of other analgesic drugs.

It is presumed that analgesic drugs act by forming some sort of barrier to the effects of the nerve impulses conveyed by the pain pathways, and the site of action might be speculatively localized in the thalamus or the cortex. At present it would be hazardous to try to suggest the mode of action of analgesic drugs. There is, for example, nothing characteristic about the influence of these drugs on the metabolism of nervous tissue.

Chemical Structure of Analgesic Drugs

Bergel and Morrison (1948) have reviewed this subject with particular reference to the relation between chemical structure and analgesic action. The structural formula of morphine is still not completely known in its three-dimensional arrangement, but it may be represented two-dimensionally as below. The complete morphine molecule has never been synthesized, but recently Grewe (1946) prepared a compound called N-Methylmorphinan which contains 4 of the 5 cyclic rings of the morphine molecule. Its formula is :—

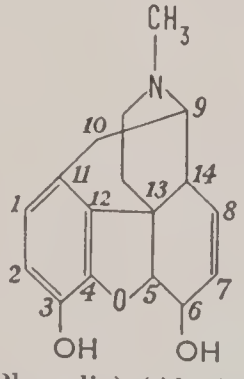


N-Methylmorphinan

Its analgesic potency is about equal to that of morphine.

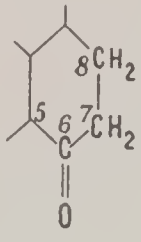
A derivative, 3-hydroxy-N-methylmorphinan hydrobromide (Nu 2206, Hoffman La Roche), has been studied by Gross *et al.* (1949). They found it to be about three times as potent as morphine in man and stated that it did not produce euphoria.

Morphine and its Derivatives

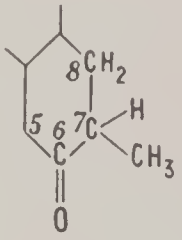


(Phenolic) (Alcoholic)

Morphine



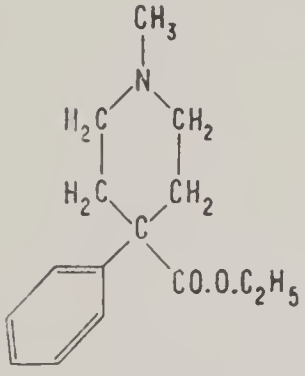
Dilaudid



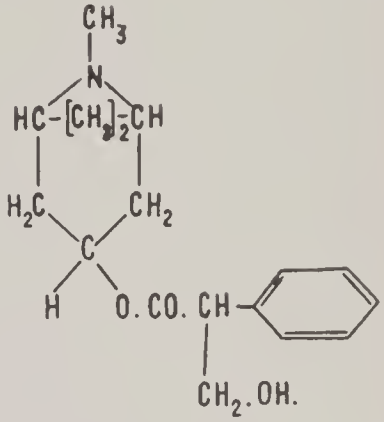
Metopon

The alkaloid morphine is a complex polycyclic compound, which has often been described as a phenanthrene derivative. The formula is presented here in a form to suggest its possible relation to pethidine and amidone. The morphine molecule is modified synthetically to produce *dilaudid* (dihydromorphinone) and *metopon* (methyldihydromorphinone).

Pethidine and Allied Compounds



Pethidine



Atropine

Pethidine (1-methyl-4-phenylpiperidine-4-carboxylic acid ethyl ester) was first studied by Eisleb and Schaumann (1939) as a spasmolytic drug of the atropine type. Its chemical relation to atropine is shown in the above formulæ. Pethidine was subsequently found to be an analgesic.

β-Pethidine is 1-methyl-3-phenylpiperidine-3-carboxylic acid ethyl ester. It has been studied experimentally by Macdonald *et al.* (1946).

β-pethidine has been found in man to be rather less potent and to have a shorter duration of action than pethidine. Its toxicity is low (Glazebrook and Branwood, 1945).

Bemidone. This compound, formed by introducing a m-hydroxyl group into the phenyl ring of pethidine, was produced in Germany during the war. Macdonald *et al.* (1946) found that its analgesic potency was about equal to that of pethidine.

Ketobemidone is 1-methyl-4-(3-hydroxylphenyl)-4-piperidylethyl ketone. It is claimed to be ten times as potent as pethidine and Kirchof (1948) reported very few side-actions from its therapeutic

use. It is a more powerful spasmolytic than pethidine (Lewis, 1949).

Other piperidine derivatives have recently been studied by Randall and Lehmann (1948) in rats, and Gross *et al.* (1948) have investigated the actions of three of these compounds in man :—

Compound *Nu* 1196 is *cis*-1: 3-dimethyl-4-phenylpropionoxy-piperidine.

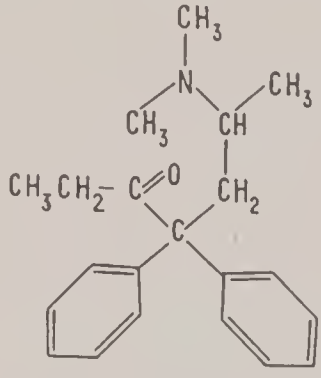
Compound *Nu* 1779 is *trans* 1: 3-dimethyl-4-phenylpropionoxy-piperidine.

Compound *Nu* 1932 is 1-methyl-3-ethyl-4-phenyl-4-propionoxy-piperidine.

These compounds are comparable in potency to morphine in rats, but clinical observations on the use of Nu 1196 in patients with pain due to cancer have shown this drug to be definitely weaker than morphine (Houde *et al.*, 1948).

Isbell (1949) has obtained evidence in man of addictive properties with bemidone, keto-bemidone (particularly marked), and the phenyl propionoxy piperidine derivatives.

Amidone and Related Compounds



Amidone

(dl-2-dimethylamino-4 : 4-diphenyl-heptan-5-one hydrochloride)

Thorp *et al.* (1947) have shown that the analgesia and respiratory depression of dl-amidone are due to the *l*-compound. Other compounds of this group studied by Thorp *et al.* include :—

Iso-amidone (dl-2-dimethylaminoethyl-3 : 3 diphenyl-hexan-4-one-hydrochloride).

"*Hoechst* 10582" (dl-1-dimethylamino-3 : 3-diphenyl-hexan-4-one hydrochloride); Ofner *et al.* (1949) have described the

preparation and actions of the compound 6-piperidino-4 : 4-diphenyl-5-methyl-3-hexanone (the piperidyl analogue of *iso*-amidone).

Phenadoxone (dl-2-1 morpholino-4 : 4 diphenylheptan-5-one hydrochloride) also belongs to the same group.

Isbell and Eisenman (1948) have shown that both l-amidone and *iso*-amidone are potential drugs of addiction, but that d-amidone and dl-2-dimethylamino-4 : 4-diphenyl-heptan-5-ol are not.

Methods for Evaluating Analgesic Drugs

Much attention has been devoted in recent years to methods for comparing the actions of analgesic drugs in animals and in man. This subject has been reviewed by Goetze *et al.* (1943) who discuss very fully the criteria for the choice of stimulus to elicit pain, and the selection of the most suitable receptive field. More recent reviews in this field include contributions by Pfeiffer *et al.* (1948) and by Miller (1948).

The nature of the response to the stimulus is important. Human subjects can describe the onset of a sensation of pain, but in animals some easily observed reflex response must be selected as an endpoint. For example, Thorp (1946), using a modification of Hardy's radiant heat apparatus for stimulation of the rat's tail, regarded slight movements of the tail or quivering of the body of the rat as a positive response.

Analgesic Drug Tests in Animals

Studies on the potency of analgesic drugs in animals have been reported by many workers, e.g. D'Amour and Smith (1941), Ercoli and Lewis (1945), Woolfe and Macdonald (1944), Thorp (1946), Davies *et al.* (1946) and Hougs-Olsen (1949). All these workers, and others, have regarded heat as the most accurately measurable stimulus. Comparisons of equianalgesic doses of the more powerful analgesic drugs show that these tests correlate quite well with clinical observations. For example Woolfe and Macdonald (1944) found in mice that morphine was about seven times as active as codeine and about half as active as diamorphine. In rats, Ercoli and Lewis (1945) recorded a similar relation for morphine and codeine, while Thorp (1946) found that diamorphine was nearly seven times as potent as morphine. These results show

that such methods are suitable for preliminary investigations on new compounds; Woolfe and Macdonald, and Ercoli and Lewis found pethidine about 1/5th as potent as morphine, and Thorp *et al.* (1947) showed that amidone was about as powerful as morphine.

The mechanism by which morphine inhibits the reflex withdrawal of the rat's tail when stimulated by heat, has been studied by Raventós (1949), who found that this drug had no such depressant action in rats after high section of the spinal cord. From the results of experimental section of the brain stem at different levels, and from the finding that central nervous stimulants such as amphetamine and caffeine could also prolong the reaction time of the withdrawal reflex, Raventós suggests that morphine depresses this reflex by stimulating an inhibitory centre situated in the rostral third of the pons.

It should, however, be added that many of these animal tests reveal very little or no analgesic action with substances such as acetylsalicylic acid or amidopyrine, which have such striking effects on certain types of pain in man. This discrepancy is probably due to a different mode of action (as compared with morphine).

Tests of Analgesic Drugs in Man

The methods employed to study and compare the effectiveness of analgesic drugs in animals make use of some reflex muscular response as an indicator of pain sensation. In man the verbal description of pain with respect to its presence, localization, intensity and character adds evidence not available in animal studies, and since the ultimate interest in analgesic drugs relates essentially to their therapeutic efficacy, human investigations are the most valuable of all. Most of the methods for testing analgesic drugs in man involve the measurement of the strength of some stimulus applied to a readily accessible part of the body, usually the skin. The smallest intensity of stimulus required to elicit a sensation described as "painful" is termed the *threshold*, and tests have been devised to measure with great accuracy the strength of stimulus employed. Macht *et al.* (1916) used a faradic current applied to the skin, Seevers and Pfeiffer (1936) measured the response to graded pressure with von Frey's hairs or needle pricks, and Hardy *et al.* (1940) applied radiant heat to the

blackened skin of the forehead. (This last method is probably the most accurate for measuring the physical intensity of stimulus.) Goetze *et al.* (1943) measured the threshold strength of an alternating current applied to a dental filling, and Harrison and Bigelow (1943) recorded the number of ischæmic muscle contractions in the forearm required to produce the typical pain, which in Lewis' classification is "deep" as opposed to the "superficial" pain arising from the skin. In all these instances the administration of recognized analgesic drugs raises the pain threshold, i.e. increases the strength of stimulus necessary to elicit the sensation of pain, but Pfeiffer *et al.* (1948) have shown that different types of analgesic drugs may affect various types of pain to different degrees.

Radiant Heat Method

The most comprehensive investigations of skin pain threshold have been those of Hardy *et al.* (1940) with the radiant heat method. The radiation from a 1,000 Watt lamp is focused on to the blackened skin of the forehead (3 sec. exposure every 30 sec. to an area of 3.5 sq. cm.). The end point is a "sensation of heat drawing together just at the end of the three second exposure, giving a quick, sharp, stinging sensation, distinct from the sensation of heat" (Hardy *et al.*, 1943). The intensity of radiation can be directly measured by means of a radiometer.

Wolff *et al.* (1940), with themselves as subjects, measured the pain threshold raising actions of injected morphine. With doses from 0.5 mg. to 15 mg. of morphine sulphate the threshold rise was linearly proportional to dosage. The following increase in threshold occurred with therapeutic doses :—

10 mg. (1/6 gr.) produced a 60 per cent increase
15 mg. (1/4 gr.) produced a 70 per cent increase
30 mg. ($\frac{1}{2}$ gr.) produced a 100 per cent increase.

With 100 per cent rise in threshold the intensity of heat was just sufficient to cause injury to the skin.

The *peak effect* of therapeutic doses occurred $1\frac{1}{2}$ 2 hours after injection. The *duration of action* after therapeutic doses was 6 7 hours.

The radiant heat pain threshold method of Hardy *et al.* has been widely used in the United States for the comparison of new

analgesic drugs with morphine. For example, Batterman and Himmelsbach (1943) found that 125 mg. of pethidine raised the threshold as much as 17 mg. of morphine, and Christensen and Gross (1948) recorded the following equianalgesic doses: 5 mg. amidone = 15 mg. morphine sulphate = 150 mg. pethidine hydrochloride. But the widespread use of this method raises the question of its limitations and inadequacies:—

Firstly, the results obtained by different workers with a single drug are by no means consistent. For example, the following differences in threshold raising action of morphine sulphate have been recorded:—

Dose of Morphine Sulphate	*Wolff et al.* (1940)	*Christensen and Gross* (1948)
5 mg.	32% rise in threshold	8% rise in threshold
10 mg.	60% ,, ,,	17% ,, ,,
15 mg.	70% ,, ,,	23% ,, ,,

It is clear that there must be some difference in the interpretation of the end-point between these two groups of workers.

Secondly, the pain threshold may be influenced by numerous psychological factors which have been carefully studied by Wolff and Goodell (1943). They found that auto-suggestion could raise the pain threshold by up to 20 per cent, that distraction produced by counting or the clanging of a bell could raise the threshold by up to 40–45 per cent, as also could shallow hypnosis. It was also shown that pharmacologically inert substances, e.g. lactose in capsules, could sometimes raise the threshold as much as 0.6 G. of acetylsalicylic acid, and that such responses, whilst more readily produced in suggestible inexperienced persons, occasionally occurred even in stable subjects thoroughly versed in the method of recording pain sensation. It would therefore appear that analgesic drugs should not be compared at a dose level which raises the pain threshold by less than 30–35 per cent, unless very careful precautions are taken to eliminate the complicating psychological influences. It is interesting to note that Sonnenschein and Ivy (1949) were unable to demonstrate that the antipyretic-analgesic drugs, e.g. aspirin, had any significantly greater influence than lactose on electrically induced pain in the tooth-pulp in man, and they suggest that these drugs act peripherally on the processes producing pain.

Thirdly, the pain threshold raising action of morphine, as tested by radiant heat on the skin of the forehead, is greatly reduced or even abolished by the presence of pain in some other part of the body. For example, if severe pain be produced by ischæmic muscular contractions before the injection of morphine, the skin pain threshold raising action of the drug is completely prevented (Wolff *et al.*, 1940). It is therefore concluded that morphine acts largely in other ways to give relief from pain, and Wolff and his colleagues have discussed these possibilities. They point out that the experience of pain may be regarded from two aspects, *pain perception*, and a *reaction pattern to pain* which is what characteristically distinguishes pain from other sensations. The reaction pattern involves withdrawal, fear, flight or anxiety and is associated with visceral reactions, this pattern being independent of pain perception *per se*. Wolff *et al.* (1942) measured the "alarm" reaction in man by recording changes in electrical resistance in the skin of the hand in response to the application of heat to the forehead. The "alarm" threshold varied widely in conditions in which the pain threshold remained constant, and if the former be regarded as a measure of the reaction pattern to pain, it certainly responded quite differently from the pain threshold. Both thresholds were raised by alcohol, the "alarm" threshold to a much greater extent than the pain threshold.

Wolff *et al.* (1940) suggest that morphine acts to relieve pain in the following ways :—

(1) It raises the pain-threshold and interferes with pain perception.

(2) It detaches the perception of pain from the fight-flight anxiety response. Pain is still perceived and recognized as pain but the usual reaction pattern to pain does not occur.

(3) It induces lethargy and sleep.

Ischæmic Pain Method

Another comment that might be made concerning the tests just described is that the induced pain is very transient, and therefore unlike the more enduring types of pain for which morphine is so frequently administered; moreover an analgesic drug is more commonly given to relieve existent pain than to prevent its development. To take account of these facts Hewer and Keele (1948) investigated the actions in normal persons

of analgesic drugs on maintained ischæmic muscular pain, the intensity of which was assessed subjectively in units from 1–10 (1=very slight, 10=intolerable pain). The drugs tested were injected intravenously when pain was moderate (4–5 units), and effective doses produced rapid transient relief (Fig. 26, amidone). It was shown that control injections of 0.9 per cent NaCl solution or subthreshold doses of analgesics were consistently ineffective. In order to compare the potency of different analgesics, the smallest dose of each drug which reduced pain of 4–5 units

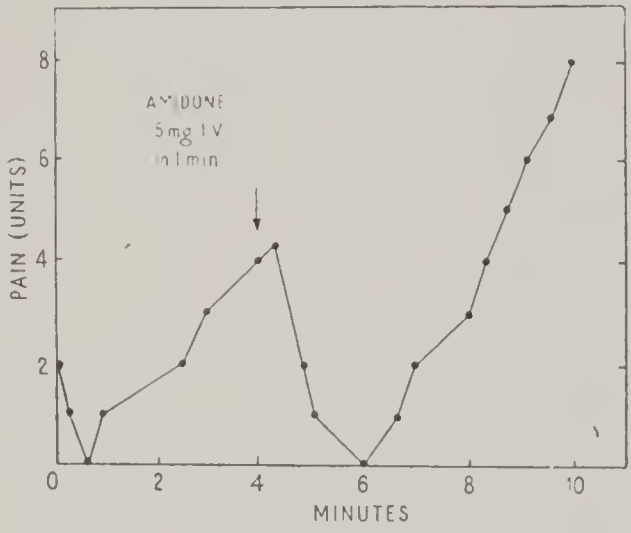


FIG. 26

INTRAVENOUS AMIDONE ON ISCHÆMIC MUSCLE PAIN

Pain is expressed in arbitrary units (see text).

(After Hewer and Keele, 1948.)

intensity was determined, and the threshold values were thus established. The results showed that morphine and amidone were about equally potent, and that both were 10–30 times as powerful as pethidine.

The maintained ischæmic pain method has also been used to demonstrate the site of action of *intravenously injected procaine* as an analgesic. It has been suggested that procaine administered in this way might be concentrated in tissues where there was increased capillary permeability resulting from burns or trauma,

but Hewer and Keele showed that procaine could relieve pain arising from ischæmic muscle, into which it certainly could not penetrate, so a central nervous action must account, at least in part, for the analgesia produced by intravenous injection of this drug.

Clinical Studies of Analgesic Drugs

Studies of pain threshold responses in animals, and even in man, are only preliminaries to the important field of therapeutic application to the relief of pain in disease. The reaction patterns of patients to pain associated with disease are naturally much more complex than the responses of normal volunteers who know that the duration of any test is limited, and that they can terminate the unpleasant experience at will. With these points in mind it may, however, still be possible to arrange conditions so that the clinical effectiveness of two analgesic drugs may be compared. The factors to be considered most carefully are the great variability in the natural course of intensity of pain in different subjects, the difficulty of comparing one patient's experience of pain with another's, and the psychological factors which so readily influence this experience.

Lee (1942) has recorded very extensive data on the comparative effectiveness of morphine, metopon and desomorphine given to patients with acute or chronic pain. He recorded the smallest effective dose for each drug and classified the effectiveness in terms of no, slight, fair or complete relief. The intensity and duration of action, the occurrence of sleep and the development of side effects were noted. Control injections of inert substances were given at intervals, and the solutions for injection were made up so that neither the nurse nor the patient knew the identity or dose of the drug. At intervals the drugs were withdrawn for 24 hours and sterile 0.9 per cent NaCl solution injected to test the possible development of the abstinence syndrome. From these data Lee concluded that 5 mg. of metopon was equivalent to 10 mg. of morphine and that these doses were equivalent to 1 mg. of desomorphine.

Hewer *et al.* (1949) have also investigated the clinical assessment of analgesic drugs. They have made use of the "pain chart" (Keele, 1948) for recording in the patient's own words the intensity of pain at regular intervals (usually hourly) throughout the day

and night. The pain is described as none, slight, moderate, severe or very severe, and the records obtained demonstrate very clearly the influence of analgesic drugs on the course of pain (Fig. 27, amidone). Hewer *et al.*, have also studied methods for summarizing their data derived from individuals with chronic pain in whom several drugs were investigated very fully, or from groups of patients in which the individuals (with more acute varieties of pain) were given only small numbers of injections. In each case the intensity of pain, as described in words, was converted to a scale of units as follows: no pain=0 units, slight pain=1 unit, moderate pain=2 units, severe pain=3 units, very severe pain=4 units. The figures recorded from individual pain charts were added together and could then be analysed statistically.

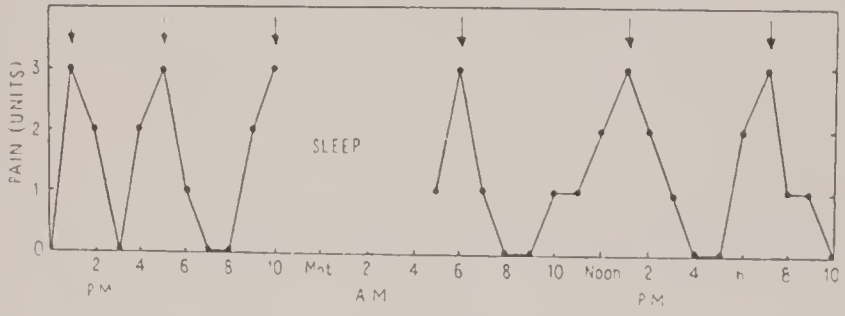


FIG. 27

Pain chart showing effects of amidone on pain in a patient with carcinoma of cervix uteri. Each arrow indicates administration of amidone of 7.5 mg. by mouth.

(After Hewer *et al.*, 1949.)

Asher (1948) has made ingenious use of different coloured tablets for the clinical comparison of analgesic drugs which may be given by mouth; he showed that inert tablets gave some relief in 50 per cent of cases.

Evaluation of Addiction Potentialities of Drugs

The most dangerous toxic action of the powerful analgesic drugs is their liability to produce addiction. One component of this state is *Euphoria* which is a feeling of well-being perhaps greater than that attained even at the peak of normal health. Euphoria can occur in normal subjects as well as in patients or in addicts, and its production by any drug is suggestive of capacity for addiction.

Another important element of the addiction picture is what is called *Psychic Habituation*, but even more outstanding and much more characteristic, is the state of *Physical Dependence*. Habituation is the craving for the drug, while physical dependence expresses an acquired bodily need for it to prevent the development of physiological disturbances mainly related to the autonomic and central nervous systems. The addiction-producing analgesics also lead to *Tolerance*. When a drug to which a person is addicted is suddenly withdrawn an *Abstinence Syndrome* develops. All these aspects of drug addiction produced by analgesics have been particularly studied in the U.S.A., where the addiction problem is much greater than in this country, and the relations between euphoria, analgesia and physical dependence were discussed in a symposium to which Oberst *et al.* (1943) contributed. Isbell (1948) has discussed this subject with particular reference to the newer synthetic analgesic drugs.

Kolb and Himmelsbach (1938) and Himmelsbach (1941) have

TABLE 8

Scoring for Evaluation of Abstinence Syndrome Intensity.

Signs	Points
Yawning	1
Lacrimation	1
Rhinorrhœa	1
Perspiration	1
Anorexia : 40% Decrease in Caloric Intake	3
Gooseflesh	3
Dilated Pupils	3
Tremor	3
Restlessness	5
Emesis : Each Act of Emesis observed	5
Fever : Each 0.1°C. rise in Rectal Temperature over Addiction Mean	1
Hyperpnœa : Each Respiration per minute increase over Addiction Mean	1
Systolic Blood Pressure: Each 2 mm. Hg. rise up to 30 mm. over Addiction Mean	1
Weight : Each pound lost from last Addiction Day	1

Abstinence Syndrome Intensity = Sum of points scored.
Critical Level = 16 points.

introduced a system of scoring for evaluating the intensity of the abstinence syndrome. The responses to drug withdrawal and the allocation of points for each factor are illustrated in Table 8. The detailed time courses of some of the changes are shown in Fig. 28.

In studying the possible addictive properties of a new analgesic drug four methods of testing may be used (Isbell, 1948) :—

(i) Administration of single doses for the detection of euphoria.
(ii) The effect of single doses on the morphine abstinence syndrome.
(iii) Substitution of the new drug for morphine in patients strongly addicted to morphine.
(iv) Direct or primary addiction to the new drug.

These points will be illustrated later in relation to pethidine and amidone.

Further, as Batterman (1948) has stressed, "the addiction liability of the new analgesic drugs must be considered from the point of view of the individual as well as the drug." The great majority of drug addicts are psychopathic subjects.

Vogel *et al.* (1948) have discussed the comparative liabilities of the powerful analgesics to produce addiction and Table 9 summarizes the position with regard to the most widely used members of this group (4 = the maximal liability in each category).

TABLE 9

Comparative Addiction Liability of Analgesic Drugs.

Drug	Physical Dependence Liability	Habituation Liability	Total
Morphine	4	4	8
Dihydromorphinone Hydrochloride	4	4	8
Metopon	3	4	7
Pethidine Hydrochloride	2	3	5
Amidone	1	4	5
Codeine	2	1	3

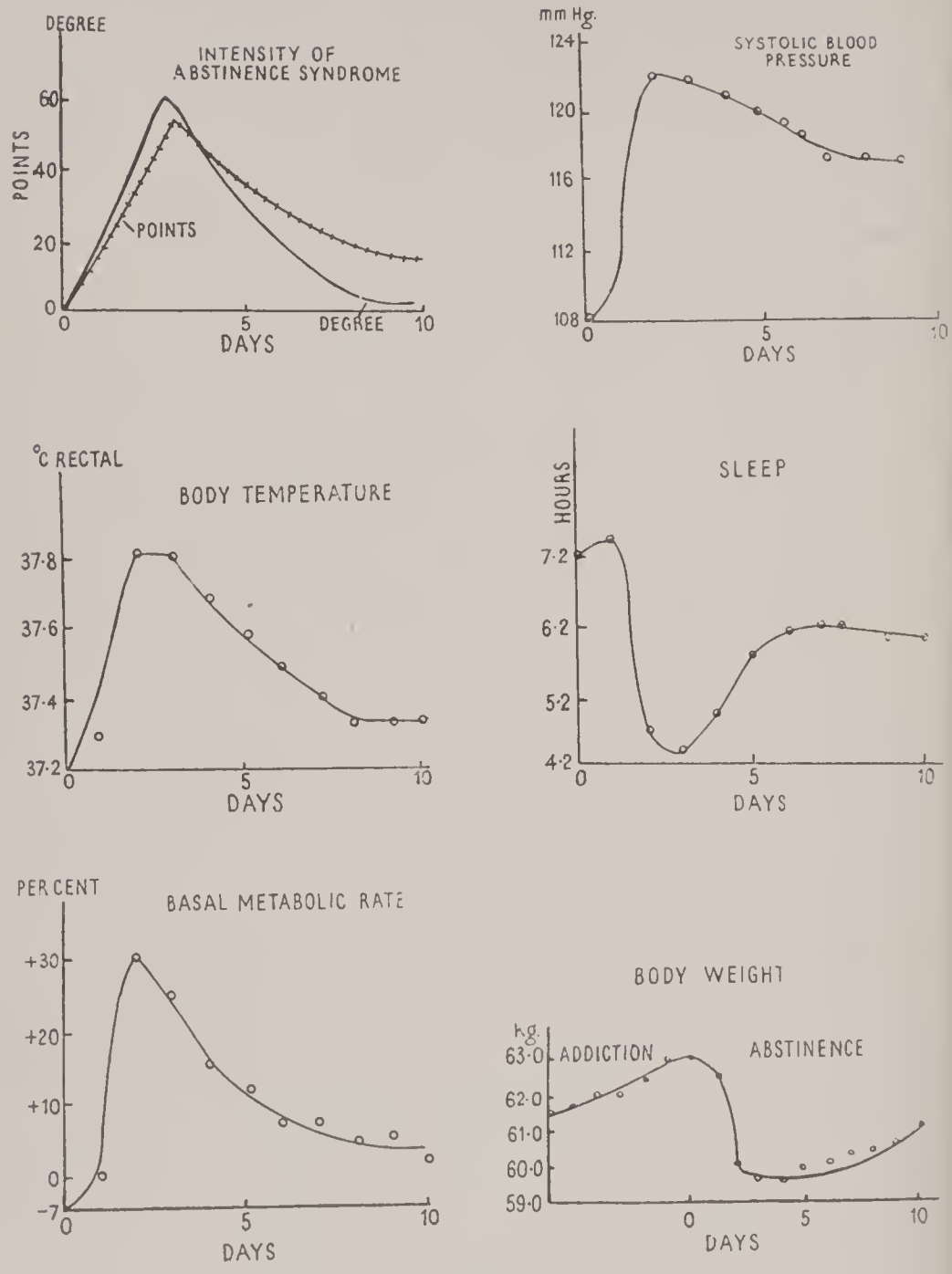


FIG. 28

MORPHINE ABSTINENCE SYNDROME

The effects of withdrawal of morphine on various body responses are shown. *Abscissa* = No. of days after abrupt withdrawal.

(After Himmelsbach, 1941.)

It will be noted that diamorphine (diacetylmorphine) is not included in this Table. It is of course even more liable than morphine to cause addiction, and for this reason its use has been abandoned, and its manufacture forbidden in the U.S.A. Monoacetylmorphine on the other hand produces only moderate euphoria (Jones and Chapman, 1944) and since it is a more potent analgesic than morphine, and is less liable to cause nausea and vomiting, it would be interesting to know more about its therapeutic actions, especially its capacity to produce addiction.

Newer Analgesic Drugs

The actions of some of the newer analgesic drugs will now be discussed. The reader is recommended to consult the reviews by Tainter and Buchanan (1949) and by Van Dyke (1949), as well as the symposium in the Annals of the New York Academy of Sciences (November, 1948) for further details.

Pethidine

Pethidine (Dolantin, Demerol, Isonipecaine, Meperidine) was originally introduced by Eisleb and Schaumann (1939) as a spasmolytic drug, but during the course of toxicity studies in mice a morphine-like erection of the tail, due probably to stimulation of the spinal cord, was observed (Straub phenomenon), and subsequently it was shown that pethidine has analgesic properties.

Pethidine is definitely less depressant to *respiration* than morphine, both in intensity of effect and duration of action. Its action on the *circulation* is slight, though intravenous injection of large doses may produce peripheral vasodilatation with a fall in blood pressure; on the *heart* it has a quinidine-like action and depresses the excitability of the muscle in response to electrical stimuli. Robbins (1945) showed that pethidine prevented the cardiac irregularities induced in dogs by cyclopropane.

Pethidine also has weak *atropine*-like effects, such as the production of mydriasis, inhibition of salivary secretion and some anti-vagal actions. It has direct papaverine-like actions on the smooth muscle of the bronchioles, intestine, uterus and blood vessels. In intact dogs pethidine produces contraction of the smooth muscle of the intestine (Gruber *et al.*, 1941), but in man Yonkman *et al.* (1944) quote Barlow as having shown that 50–100 mg. of pethidine intramuscularly reduced or abolished the motility

of the gastro-intestinal tract for up to 1½ hours. Although gastric and colonic tone are reduced by pethidine, biliary pressure is increased, probably from spasm of the "sphincter" of Oddi (Gaensler *et al.*, 1948). Indeed these authors found that pethidine was nearly as powerful as morphine in this respect, and in two patients they recorded the production of attacks of biliary colic after injection of pethidine. As with morphine, such spasm can be relieved by nitrites, aminophylline or adrenaline, but not by atropine or papaverine.

The ureter behaves differently, and Climenko and Berg (1943) found that pethidine reduced the amplitude of contractions and the tone of the ureter in dogs and in man.

Pethidine is also a *local anæsthetic* and Way (1946) showed in tests on the rabbit's cornea, the frog's sciatic nerve, and on intradermal injection in man, that it possessed about 70 per cent of the activity of cocaine, but it is not suitable for clinical use since it produces preliminary irritation.

Absorption, Distribution, Fate and Excretion of Pethidine

Way *et al.* (1949) have investigated the absorption and distribution of pethidine in rats (using Brodie's methyl orange method). After oral administration over 90 per cent absorption from the gut had occurred within 4 hours. Studies of distribution showed the highest concentrations in the kidneys, spleen, lungs and liver and moderate concentrations in the brain.

Lehman and Aitken (1943), Oberst (1943) and Way *et al.* (1949) have studied the excretion of pethidine and recovered 5–20 per cent in the urine both after oral administration and after subcutaneous injection of the drug in man. It would thus appear that over 80 per cent is destroyed in the body, and the work of Bernheim and Bernheim (1945) suggests that pethidine is hydrolysed in the liver by an esterase distinct from tropine esterases, cholinesterases and the esterases which hydrolyse aliphatic esters. Way *et al.* (1947) found that after partial hepatectomy in rats, the actions of pethidine, and its level in the blood, were greater than in normal animals, and they suggest that the effects of pethidine in man might be enhanced by liver disease.

Way *et al.* (1949) have also recovered pethidine from saliva in man, but found none in the milk of lactating mothers within 1–6 hours of injection of the drug (100 mg.). They also recovered small

amounts of pethidine (less than 1 per cent of the dose injected into the mother) from the urine of new-born infants.

Pethidine and Addiction

The development of addiction to pethidine was reported soon after its introduction in Germany, and more extensive observations in other countries have since confirmed its capacity to lead to tolerance, habituation and physical dependence. Himmelsbach (1942) has studied this question from different angles :—

(1) In morphine addicts stabilized on the smallest dose of morphine required to prevent abstinence symptoms, the substitution of pethidine for morphine allowed some withdrawal signs to appear.

(2) After a week's administration of pethidine alone (as a morphine substitute), abrupt withdrawal produced a smaller abstinence syndrome than after withdrawal of morphine (Fig. 29).

(3) In four subjects repeated injections of pethidine produced addiction, which after 1–2 months was followed by a very mild withdrawal syndrome, whereas after 10–11 weeks administration the withdrawal effects were quite marked, though still less than those of morphine.

Yonkman *et al.* (1944) noted symptoms such as nausea, vomiting, sweating, tremors, nervousness, irritation and depression some hours after abrupt withdrawal of pethidine in nine patients, but it is generally agreed that the withdrawal signs and symptoms are not very severe, and occasionally, as in a case described by Curry (1947) where the dose of pethidine rose finally to 1,250 mg. daily, there were no abstinence signs whatever on withdrawal of the drug.

With regard to the development of tolerance to pethidine, Wieder (1946) recorded an increase in dose up to 2–3 G. daily in two patients, and Polonio (1947) describes three cases in whom 5, 7–8 and 12–14 G. respectively, were taken daily. These high doses were liable to cause tremors, muscular twitching in the extremities, muscular rigidity, exaggerated reflexes and even petit mal-like seizures. Andrews (1942) found that doses up to 4.8 G. daily produced slow large waves in the electroencephalogram similar to those seen in some types of epilepsy.

To summarize the points concerning pethidine and its liability to cause addiction :—

(1) Pethidine can produce euphoria.

(2) It is a partially effective substitute for morphine in morphine addicts.

(3) It can produce psychic dependence (habituation).

(4) Tolerance can be acquired to pethidine.

(5) Physical dependence can occur. The withdrawal syndrome is less severe than with morphine.

(6) Addiction is much more likely to arise after injection than after oral administration.

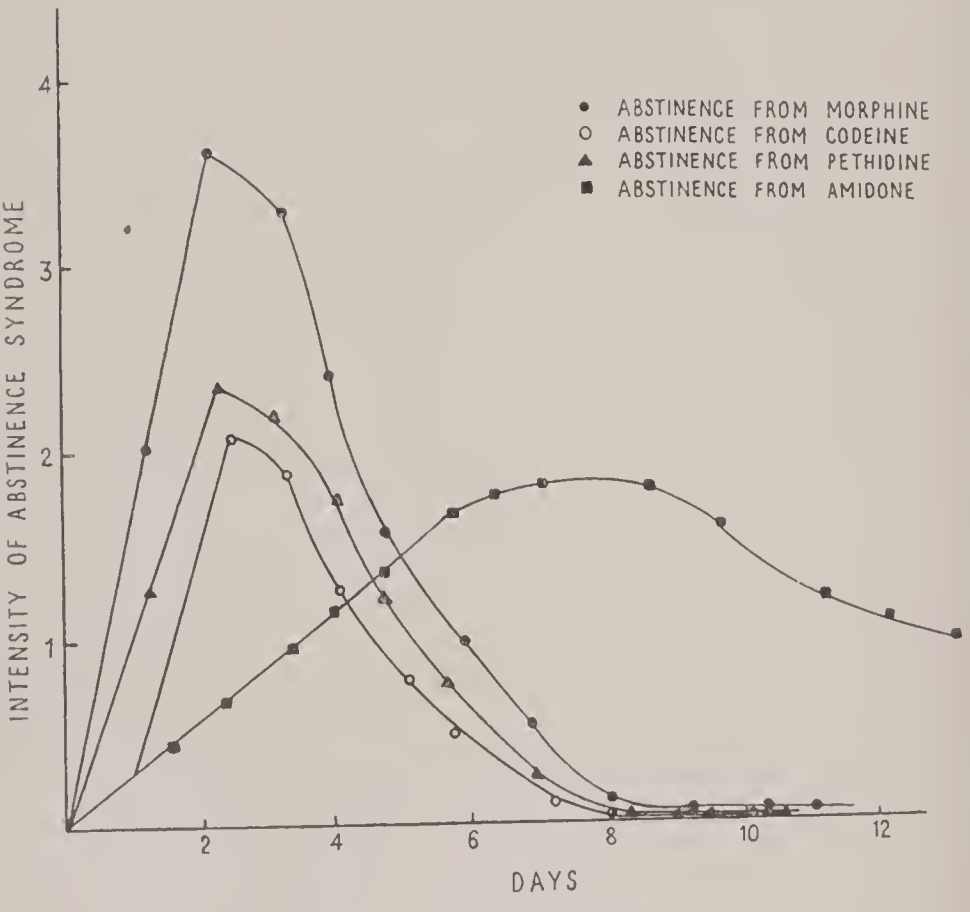


FIG. 29

INTENSITY OF ABSTINENCE SYNDROME

The intensity of the abstinence syndrome after withdrawal of morphine, codeine, pethidine and amidone, is expressed in arbitrary units. The intensity is greatest with morphine, and the duration is greatest with amidone.

(After Vogel *et al.*, 1948.)

(7) Pethidine must be regarded as a drug of addiction. It has been placed under the Dangerous Drugs Acts restrictions in Great Britain.

Clinical Uses of Pethidine

Accounts of the clinical use of pethidine in Germany soon followed the discovery of the drug by Eisleb and Schaumann in 1939. More recent and detailed reports on the general use of pethidine (its use as an obstetric analgesic is considered separately) have come from Batterman and Himmelsbach (1943), Yonkman *et al.* (1944), Christie (1943), Fitzgerald and McArdle (1943) and others. The main points revealed by these studies are:—

(1) Pethidine is about 1/10 as potent an analgesic as morphine when given by intramuscular injection.

(2) Although pethidine can relieve most kinds of pain, it may be somewhat more effective against pain associated with smooth muscle spasm, owing to its additional spasmolytic action. As already mentioned, pethidine increases tone in the biliary tract and, as with morphine, any relief following its administration for biliary colic would be due to its central action.

(3) After intramuscular injection the analgesic action develops within 15 minutes and lasts 5–6 hours (the effective duration must of course depend on the intensity of pain).

(4) Oral administration of pethidine is less effective than injection, but is sometimes superior to a mixture containing acetylsalicylic acid, 0.5 G.+phenacetin, 0.5 G.+codeine phosphate 16 mg. (Christie, 1943).

(5) Pethidine has less hypnotic and sedative actions than morphine, and does not depress the cough centre.

(6) Pethidine does not cause constipation.

(7) Side effects include nausea, vomiting, dry mouth, dizziness, faintness, pallor, visual disturbances, euphoria, restlessness and anxiety. The nausea and vomiting are less than with morphine. Tolerance and addiction are discussed elsewhere (pp. 255–257).

(8) The usual dose of pethidine is 50–100 mg. by intramuscular injection or by mouth. The dose should not exceed 150 mg. 3 hourly. Occasionally intravenous injection of 50–100 mg. is used.

Pethidine in Labour

Pethidine was first used to relieve the pains of childbirth by Benthin (1940). In this country there have been reports on this use of pethidine by Gallen and Prescott (1944) in 150 cases, and more recently by Barnes (1947) in 500 labours.

The results may be summarized as follows :—

(1) Pethidine in doses of 100–400 mg. intramuscularly was an effective analgesic in the majority of cases.

(2) Pethidine was generally well tolerated by the mothers.

(3) Pethidine was not considered responsible for the deaths of any babies in either series, though it might have contributed to asphyxia in some cases.

(4) There were no significant actions on uterine contractions or on the duration of labour, and there was no tendency to post-partum haemorrhage.

(5) Pethidine did not prevent the patient from co-operating intelligently during the second stage of labour.

The combined use of pethidine and scopolamine in labour was first reported by Roby and Schumann (1943) and has recently been described by Roberts (1948) in 500 labours. The maximum total doses of the drugs within 18 hours were 400 mg. of pethidine + 1.3 mg. of scopolamine. To the analgesic action of pethidine was added the amnesic effect of scopolamine, and there was no evidence of any additional harmful effects on the mother or child, and no prolongation of labour (see also Hershenson, 1948).

It may be said that pethidine is the most satisfactory single drug for the relief of the pains of labour. Its great virtue is that analgesia occurs with only slight and transient depression of the fœtal respiratory centre, in which respect it compares most favourably with morphine and amidone. It should, however, be pointed out that pethidine is not likely to be sufficiently powerful to prevent the pain frequently associated with the later stages of labour, when the administration of nitrous oxide or trichlorethylene may be required. There is, however, some evidence that the combined use of these agents with pethidine may double the incidence of asphyxia neonatorum (Royal College of Obstetricians and Gynæcologists' Report, 1948).

Amidone

Amidone [Hoechst 10820, Physeptone, Miadone, Dolophine, Adanon, Methadon (e), Bntalgin] was introduced during the war in Germany; it has been subsequently developed, and related compounds have been studied in Great Britain and in the United States.

Analgesic Actions

Scott and Chen (1946) showed that amidone (5 mg./kg.) was a more powerful analgesic than the same dose of morphine in the dog, and in man they found 5 mg. by mouth as potent as 150 mg. of pethidine; mice showed the Straub tail phenomenon, and cats responded, as to morphine, with excitement. Thorp *et al.* (1947) found that in rats amidone was as powerful an analgesic as morphine, and that both were 7–10 times more potent than pethidine. Christensen and Gross (1948), using the Hardy-Wolff radiant heat method in man, have claimed that 5 mg. of amidone is as effective as 15 mg. of morphine, but Hewer and Keele (1948), in studies on the actions of these drugs on ischæmic muscular pain, and Hewer *et al.* (1949) in their clinical investigations, found amidone and morphine about equally active.

The other actions of amidone have been reviewed by the Council on Pharmacy and Chemistry (1948). They may be summarized thus:—

(1) Amidone is less liable than morphine to produce nausea and vomiting, except when it is given by mouth.

(2) Amidone produces less constriction of the pupil than morphine.

(3) Amidone depresses breathing less than does morphine, but more than pethidine (Thorp *et al.*, 1947). Prescott and Ransome (1947) have concluded that the depressant action on the infant's respiratory centre contra-indicates the use of amidone in obstetric analgesia. Amidone also depresses the cough reflex.

(4) Amidone is much less constipating than morphine. Uchiyama *et al.* (1947) found that on the isolated rabbit gut and rat uterus, amidone was nearly as powerful a spasmolytic as pethidine. In intact animals intestinal motility may be increased by central vagal stimulation. Amidone does not cause urinary retention.

(5) Amidone in the usual doses is not a sedative or hypnotic, and should not be used where the sedative effect of morphine is required, e.g. in pre-anæsthetic medication.

(6) Therapeutic doses have no significant actions on the circulation. Large doses intravenously may produce bradycardia and a fall in blood pressure.

(7) Amidone has a local anæsthetic action. It is also a weak histamine antagonist (Chen, 1948).

(8) *Side Effects. Euphoria* is unusual with doses of the order of 5–10 mg., but it may occasionally occur, and has been recorded in former morphine addicts, who should know. Other side-effects include nausea and vomiting, lightheadedness and giddiness, which occur more frequently in ambulant patients than in those confined to bed. Glazebrook (1949) found that side effects were no less frequent with amidone than with morphine.

Batterman and Oshlag (1949) have stated that mental confusion and toxic psychosis may occur when amidone is given for prolonged periods to patients over 50.

(9) *Tolerance.* Isbell *et al.* (1947) administered very large doses of amidone, e.g. 60 to 100 mg. q.i.d. for 28 to 180 days to 15 subjects, and noted the development of tolerance to its analgesic and sedative actions. There have, however, been no reports of increased tolerance after prolonged administration of therapeutic doses.

(10) *Physical Dependence.* Amidone was found by Isbell *et al.* (1947) to be an effective substitute for morphine in morphine addicts.

(i) It relieved the abstinence symptoms which followed the abrupt withdrawal of morphine in addicts.

(ii) Amidone was effective in preventing the appearance of withdrawal symptoms when substituted for morphine in stabilized addicts. The adequate dose of amidone was about 1/4 that of the previously administered morphine.

(iii) In addicts who had been stabilized on amidone, abrupt withdrawal of the drug produced a characteristic abstinence syndrome which differed from the morphine withdrawal picture in developing more slowly, in being less intense and in lasting longer. Fig. 29 shows a comparison of the amidone

abstinence syndrome with that of morphine, codeine and pethidine.

Woods *et al.* (1947) have studied the possibilities of amidone addiction in rhesus monkeys, which can readily become addicted to morphine. They found that prolonged administration of amidone did not produce tolerance or physical dependence.

Since amidone can occasionally produce euphoria, and since it can so effectively substitute for morphine, it must clearly be regarded as a possible drug of addiction. In Great Britain its use is restricted by the Dangerous Drugs Acts.

(11) *Administration.* Amidone may be given *orally* in doses up to 7.5 mg. 3 or 4 hourly, or *subcutaneously* in single doses of 2.5–10 mg. To relieve cough 1.25–2.5 mg. is given by mouth.

After subcutaneous injection the analgesic action develops in 15–20 minutes, and lasts 2–4, or more, hours, depending on the dose and the intensity of pain. After oral administration the action takes ½ hour or more to appear.

Other Amidones

The name amidone usually refers to the dl- compound. Robbins (1949) has investigated the value of this and other amidones in pre- and post-anæsthetic medication, and noted that the following doses of the various compounds were equivalent to 10 mg. of morphine :—

l-amidone, 3.7 mg ; dl-amidone, 7.5 mg ;
dl-isoamidone, 10–12 mg ; l-isoamidone, 7.5 mg.

Phenadoxone (Compound "C.B.11", Heptalgin, Heptazone)

Wilson and Hunter (1948) studied the actions of phenadoxone on normal subjects with induced ischæmic muscular pain, and found that it was at least as potent as amidone and more than ten times as potent as pethidine. Clinically it was administered in doses of 10–20 mg. by intramuscular or intravenous injection, or up to 30–50 mg. by mouth. On intramuscular injection the action of phenadoxone began in 10–15 minutes and lasted 2–3 hours ; by mouth it began to act in 20–30 minutes and the effect lasted 3–4 hours. Phenadoxone had no hypnotic action. Flataker and Winter (1949) found that in dogs phenadoxone was shorter acting than amidone, and that its sedative effect was less than that of morphine or amidone. They further observed, very significantly, that

dogs tolerant to morphine were also tolerant to phenadoxone, which must certainly be regarded as a potential drug of addiction.

"Piperidyl *Iso*amidone"

This drug was introduced by Ofner *et al.* (1949), and was shown in rats to be as active an analgesic as morphine, with only 1/3 the respiratory depression of the latter. Prescott *et al.* (1949) have found that "piperidyl isoamidone" has less respiratory depressant action in man than morphine, amidone or pethidine, and it is being tried as an obstetric analgesic.

Fate and Excretion of Amidone

The metabolism of amidone in *rats* has been studied by Eisenbrandt *et al.* (1949) and by Elliott *et al.* (1949). Both groups of workers used amidone containing radioactive carbon and their findings may be summarized thus:—

(i) Amidone is completely absorbed from its site of subcutaneous injection within two hours.

(ii) The highest concentrations of the drug were found in the liver, intestine, lungs and adrenals; the concentrations in the brain were very low.

(iii) About 60% of amidone was excreted in the fæces (probably mainly via the bile) and less than 30% in the urine, within 24 hours. Scott and Chen (1946) and Cronheim and Ware (1949) have recorded similar figures for urinary excretion in *man*.

Metopon

Metopon was introduced by Eddy in 1936. Lee (1942) found that 5 mg. of metopon was as effective an analgesic as 10 mg. of morphine, that tolerance and physical dependence developed more slowly, and that it caused fewer side effects and had less hypnotic action than morphine. Lee warned against its use in premedication owing to its occasional production of dangerous respiratory depression. Metopon is more liable to produce addiction than pethidine and amidone (Van Dyke, 1949).

Eddy (1947) reported that metopon had very little emetic action. It was only partially effective as a substitute for morphine in morphine addicts. Metopon is effective by mouth and has been

used in the U.S.A. made up in capsules each containing 3 mg. The drug has been used solely for the relief of pain in cancer patients, and recently the Council on Pharmacy and Chemistry (1948) has reported on its use in general practice. The minimal effective analgesic dose in non-tolerant persons was 6 mg. (2 capsules), and the duration of action was 5–10 hours.

It should be added that metopon is prepared from thebaine, that it is expensive and difficult to make, and that it is not available in this country.

Intravenous Procaine as an Analgesic

The use of intravenous procaine to relieve the pruritus associated with jaundice was described by Lundy (1942). Its application to the relief of pain associated with burns was reported by Gordon (1943), its use in obstetric analgesia by Allen (1945), and the relief of postoperative pain was described by McLachlin (1945). The results of over 2,000 infusions of procaine in traumatic, inflammatory and other states have been presented by Graubard *et al.* (1947). They administered 0.1 per cent procaine hydrochloride at a rate of about 4 mg./kg. in 20 minutes (i.e. 14 mg./minute in a 70 kg. patient). Apart from relief of pain, the effects noted were a sensation of warmth in the whole body, flushing of the skin of the face and neck, a dry mouth, mydriasis, light-headedness and a feeling of relaxation. Larger doses, e.g. 30–60 mg./minute, produced dizziness, apprehension and drowsiness, perhaps leading to momentary loss of consciousness. Allen (1945) has described signs of central nervous stimulation, e.g. convulsive movements, with high rates of infusion and recommends the prior administration of barbiturates to prevent these effects.

The safety of intravenous procaine infusion depends on the rate of destruction of this compound by procaine esterase in the blood and liver (Kisch *et al.*, 1943 ; Burgen and Keele, 1948 ; Brodie *et al.*, 1948). Rovenstine (1948) has suggested that the analgesia produced by procaine infusions may in part be due to the diethylaminoethanol liberated on hydrolysis of procaine, but Rovenstine and Papper (1949) found that very large doses of this compound were necessary to relieve pain. Intravenous procaine has also been used in recent years to produce vasodilatation in vascular diseases, to reduce cardiac irritability in arrhythmias (by a quinidine-like action, Dawes, 1946) and for the suppression of certain allergic

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CHAPTER 13

THE HÆMOPOIETIC SYSTEM

Iron

The metabolism of iron is of interest not only with respect to the fate of iron in the diet, but also in relation to the administration of iron preparations in the treatment of iron deficiency anæmia, which is our chief concern in this section. It was formerly thought that iron was absorbed from the stomach and duodenum, and that any excess was subsequently excreted by the colon. It was, however, shown by Welch *et al.* (1936), in studies made on a young woman with an ileostomy stoma and an isolated large intestine, that there was no significant excretion of iron into the colon. McCance and Widdowson (1937 and 1938) found that, apart from a small initial transient excretion of iron in the urine, intravenously injected iron was retained by the body; there was no evidence whatever that parenterally administered iron was excreted by the large intestine. McCance and Widdowson therefore proposed the theory that the iron balance of the body is regulated entirely by control of iron absorption; it follows from this that the iron which normally appears in the fæces represents iron which has never been absorbed from the intestine. Further reports by these authors (McCance and Widdowson, 1937) on a patient with polycythæmia in whom severe hæmolysis was therapeutically induced by acetylphenylhydrazine, and (McCance and Widdowson, 1943) on another patient with very severe hæmolytic anæmia, who received numerous blood transfusions, showed that iron excretion was negligible in spite of the liberation of vast quantities of iron from the hæmolysed red cells. From this work it is clear that the control of iron absorption from the alimentary tract is extremely important.

Absorption of Iron

It is not proposed to discuss in detail the sources and types of dietary iron, or all the factors which may influence its absorption, since these aspects have been recently reviewed by Cartwright

(1947) and Hynes (1948). The older indices of iron absorption, e.g. increases in hæmoglobin and serum iron concentrations, have been reinforced in recent years by the use of radioactive iron.

Iron is absorbed from the stomach and from the small intestine (chiefly the upper part), and there is evidence that absorption can occur from the colon. Radioactive iron, when fed to dogs, appears quickly in the plasma and reaches peak concentrations in 4–8 hours, which suggests that absorption is complete in this time. Numerous factors can influence the absorption of iron :—

(1) Dissociation of iron salts is considerable in acid solutions, as in the stomach, but above pH 5.0 the dissociation is very slight, so that absorption in the small intestine will diminish rapidly beyond the duodenum. The effect of gastric achlorhydria will naturally be greater on absorption of the small quantities of iron in the diet, than on the absorption of the large doses of iron salts administered therapeutically. The action of free gastric HCl is primarily to dissolve and ionize the iron. A further point to be noted is that the iron salts brought into solution by HCl will be precipitated in the small intestine in a finely divided state, in which it will be quite readily absorbed.

(2) Substances which form insoluble compounds with iron diminish its absorption. For example, Brock and Taylor (1934) showed that phosphates, and McCance *et al.* (1943) showed that phytic acid, could reduce absorption of iron.

(3) Ferrous salts are better absorbed than ferric salts in man. This fact was well illustrated by Witts (1936) who compared the therapeutically effective doses of ferrous and ferric compounds when given to patients with hypochromic anæmia. Moore *et al.* (1939) studied the absorption of iron by determining the level of serum iron after administration of iron salts by mouth, and noted higher values after ferrous than after ferric salts. Moore *et al.* (1944), have also measured radioactive iron uptake in terms of its utilization for hæmoglobin formation. Normal men utilized 1.5–10 times as much ferrous as ferric iron, and in patients with hypochromic anæmia the ratio was 2–15 (Fig. 30). In dogs these differences are less marked.

(4) The relation between iron absorption and bodily need for iron is very interesting. Hahn *et al.* (1938) showed that in chronic hæmorrhagic anæmia in dogs, absorption of radioactive iron was greater than in normal animals. Balfour *et al.* (1942) found that in

patients with hypochromic anæmia absorption of radioactive iron was 10 times as great as in normal subjects; they also showed a greater absorption of iron during pregnancy. Hynes (1949) has shown that when the iron reserve of the body is exhausted by repeated bleedings, the utilization of dietary iron is increased to about 33 per cent (normal=7 per cent). Thus iron absorption is increased in conditions where there is an increased bodily demand for this element. (Fig. 30 illustrates this point.)

It should perhaps be added that the amount of radioactive iron present in circulating hæmoglobin is not always a reliable index

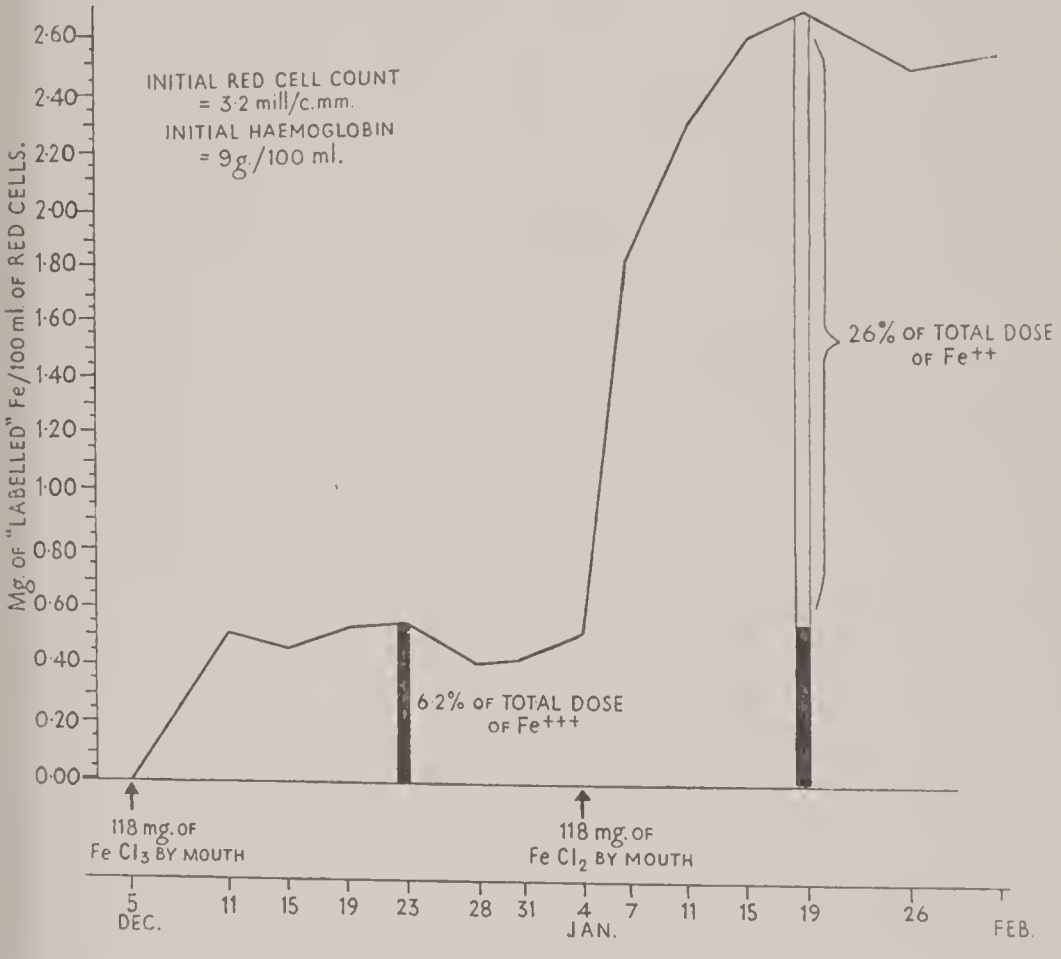


FIG. 30

Absorption of orally administered radio iron in a patient with hypochromic microcytic anæmia. Ferrous iron is better absorbed than ferric iron.

(After Moore *et al*, 1944.)

of iron absorption. For example Dubach *et al.* (1946) showed that in untreated pernicious anæmia only very small amounts of orally administered radioactive iron appeared in the blood stream. However, after a reticulocytosis had been produced by injection of liver extract, the proportion of radioactive iron found in the circulating blood increased considerably ; this suggests that in the untreated anæmia the iron was absorbed and stored somewhere in the body, but could not be utilized until the total hæmoglobin production was much increased. Dubach, Callender and Moore (1948), also found that in patients with fever or with refractory anæmias, more iron is absorbed than is used for hæmoglobin formation.

Mechanisms for Control of Iron Absorption

Hahn *et al.* (1943) studied in dogs some of the mechanisms concerned in the control of iron absorption. Whereas in chronic hæmorrhagic anæmia absorption of radioactive iron was much increased, acute blood loss *per se* had no effect on iron absorption within 24 hours ; similarly the production of anoxia for 48 hours did not increase iron absorption. They also noted that oral administration of an iron salt a few hours before the administration of radioactive iron, reduced the absorption of the latter ("mucosal block"), an observation which harmonizes with previous findings that small doses of iron are more efficiently utilized than large ones. These authors found no correlation between the concentration of iron in the gut and the serum level, and in order to explain all these facts they postulated the existence of some "iron-acceptor" mechanism in the gastric and intestinal mucosa. In normal persons the acceptor mechanism can become saturated within 1–2 hours, but desaturation takes several days. In iron deficiency anæmia the transfer of iron from the "iron-acceptor" to the plasma is accelerated, and so more iron can be absorbed from the intestinal lumen to combine with the acceptor.

Ferritin

Granick (1946 and 1947) has suggested that the above mentioned results of Hahn *et al.* (1943) can be most simply explained by the presence of *ferritin* in the mucosa of the alimentary tract, and that ferritin is also concerned with storage of iron in other organs ; its rôle in this latter connection will be discussed later.

Ferritin is a brown coloured compound formed by the union of the colourless protein, *apoferritin* (molecular weight 465,000), with small micellar units of a particular kind of ferric hydroxide; the iron content may be up to 23 per cent by dry weight of the compound. Ferritin was first isolated by Laufberger in 1937, who obtained it as a crystalline compound with cadmium sulphate; apoferritin also crystallizes with cadmium sulphate. The iron hydroxide in ferritin is curious in that it is magnetically distinguishable from iron hydroxide found elsewhere in nature; its ferric iron contains 3, instead of the usual 5, unpaired electrons in its outer shell.

Granick demonstrated the presence of ferritin in the intestinal mucosa by its formation of crystals with $CdSO_4$. In guinea pigs,

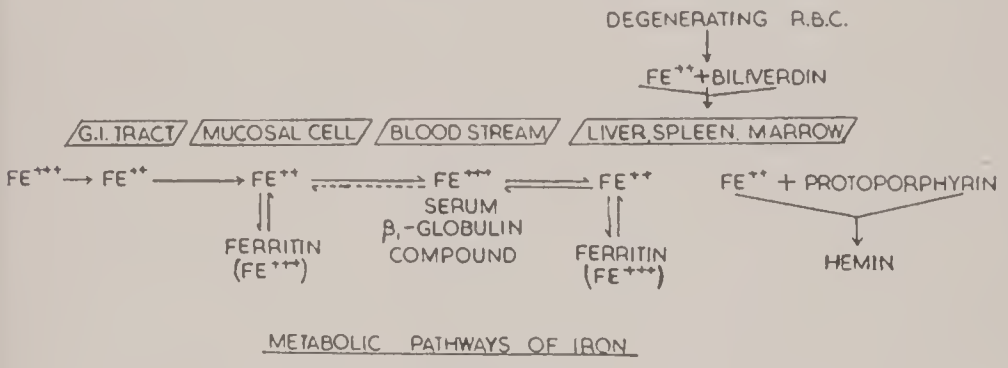


FIG. 31
(After Granick, 1947.)

ferritin was normally present only in the duodenal mucosa, and there only in traces. After feeding with iron, an increase in ferritin content was seen in the mucosa along the whole gastro-intestinal tract. In the duodenum the ferritin content reached a peak within seven hours, and decreased by the third to sixth day to normal levels. These changes may be correlated with the time-course of saturation and desaturation of the "iron-acceptor" mechanism of Hahn *et al.* (1943).

Granick has proposed the following scheme for absorption of iron (Fig. 31): "Iron is absorbed by the mucosal cells in the ferrous form. In the mucosal cells it is postulated that an equilibrium exists between the ferrous iron of the cells and the ferric iron of ferritin. In the presence of ferritin the cells are considered to be "physiologically saturated" with ferrous iron. Only when

the ferritin iron has decreased to a point where the cells are no longer "physiologically saturated" with ferrous iron would more iron be absorbed by the cell." Granick has shown further that before administration of iron there is not only very little ferritin in the mucosa, but practically no apoferritin either; it therefore appears that the absorption of iron causes the rapid formation of the specific protein to which it can become attached. When ferritin loses its iron, apoferritin also disappears.

On Granick's view the increased iron absorption in chronic hæmorrhagic anæmia would be due to the fall in the serum iron level, which in turn would allow ferrous iron to pass from the mucosal cell to the blood stream; the equilibrium with ferritin iron would be disturbed and when the concentration of ferritin iron had been sufficiently reduced, the cell would no longer be saturated with respect to ferrous iron. At this stage more iron could be absorbed by the cell from the intestinal lumen.

Other Actions of Ferritin

Mazur and Shorr (1948) have shown that ferritin is identical with the vaso-depressor material (VDM) which has been isolated from the liver and blood of dogs in irreversible shock, and have suggested from studies on the vessels of the rat's mesoappendix that the ferritin-apoferritin system is important in the regulation of the peripheral circulation. Corcoran *et al.* (1949) have, however, found that in rats intravenous injection of ferritin has no consistent effects on blood pressure or on the response to adrenaline.

Baez *et al.* (1949) have also reported that ferritin is an anti-diuretic in rabbits and dogs.

Iron Transport

Moore *et al.* (1939) showed that iron is absorbed from the gut into the blood and not into the thoracic duct lymph. They found that in normal subjects the oral administration of 10 G. of ferric ammonium citrate raised the serum iron level from 100 to 360 μg./100 ml., this peak value being reached after $4\frac{1}{2}$ hours; the serum iron level returned to the original value in 12 hours. Hahn *et al.* (1939), using oral radioactive iron in dogs, noted peak plasma iron values at 4–8 hours, with a return to normal in 24 hours. It would appear highly probable that plasma iron is iron in process of transport from the site of absorption in the gut, or from sites of

hæmoglobin breakdown, to sites of storage, or to the red bone marrow in which hæmoglobin synthesis is performed. The normal plasma iron concentration in man is 50–180 μg./100 ml.

Laurell (1947) has studied iron transport in the serum in some detail. He points out that the total amount of iron present in blood serum is about 0.1 per cent of the total store of body iron; the total serum iron therefore is about 4 mg., and since about 25 mg. of iron are required daily for hæmoglobin synthesis, considerable amounts must be transported from the sites of breakdown and storage to the sites of hæmoglobin formation in the bone marrow. Laurell has also shown that there is a "saturation limit" for the binding of iron by serum; in normal subjects about 315 μg. was the maximum amount of iron which could be bound by 100 ml. of serum. In the latter part of pregnancy the saturation limit rose to 446 μg./100 ml. Normally the serum iron level is about 1/3 of the saturation value, but in anæmias both values may vary independently; for example in chronic hæmorrhagic anæmia, the serum iron level is lowered and the saturation limit raised, whereas in pernicious anæmia the reverse occurs. The changes in infection will be discussed later.

Investigations on the nature of the plasma protein which combines with iron have been reported by Surgenor *et al.* (1949), who found that Cohn's fraction IV–7 combined with metals such as copper, zinc, and especially iron. This fraction is a β_1 *globulin* with a molecular weight of 90,000; it constitutes about 3 per cent of the total plasma protein, and has been shown by Jager (1949) to be immunologically homogeneous. Rath and Finch (1949) showed that 1 mg. of this pure β_1 globulin combines with 1.25 μg. of iron. Laurell (1947) was unable to determine whether the iron combined with this globulin was in the ferric or ferrous form, though Granick has assumed that the high O_2 tension in blood would produce ferric iron.

There is no direct passage of radioactive iron from its combination with plasma globulin into red blood cells *in vitro*, and the demonstration by Hahn *et al.* (1939) that in anæmic dogs radioactive iron appeared inside the red cells within four hours of oral administration, probably means that fresh hæmoglobin formation in the bone marrow, incorporating the radioactive iron, had occurred within that time.

Utilization of Intravenous Iron

The utilization of intravenously administered radioactive iron for hæmoglobin formation has been studied by Dubach *et al.* (1946) and by Finch *et al.* (1949). In *normal persons* the amount of radio iron in the red cells steadily increased, until about 2 weeks after injection 75 per cent was found in these cells, the remaining 25 per cent presumably having been stored in the tissues. In patients with *iron deficiency anæmia*, in whom both circulating hæmoglobin and storage iron were reduced, the initial uptake of iron was quicker and the final percentage utilization was higher. In *hæmochromatosis*, in which the circulating hæmoglobin was normal and the storage iron enormously increased, the final utilization of iron did not exceed 20 per cent, and in *aplastic anæmia*, in which the circulating hæmoglobin was reduced and the storage iron was normal, a similar figure was recorded.

Distribution and Storage of Iron

The distribution of the total body iron in dogs has been summarized by Hahn (1937):—

Table 10

Circulating Iron		Available Iron reserves		Non-available iron reserves	
Blood Hæmoglobin	57%	Liver, Spleen, Bone Marrow	15%	Myohæmoglobin	7%
		Elsewhere	5%	Parenchyma iron (cytochrome, catalase, etc.	16%
Totals	57%		20%		23%

As Hynes (1948) points out, these figures mean that the available iron reserve is about 850 mg. which is sufficient to replace about 1/3 of the circulating hæmoglobin, so that a blood loss of this order can be made good, in a healthy subject, without need for administration of additional iron. However, Hynes (1949) has since

reported an experiment in which a subject was submitted to repeated bleedings over a period of 136 days (total blood loss was 2,750 ml.). The iron reserves were in this way exhausted, and calculations showed that the reserve amounted to about 600 mg. of iron.

The iron taken up by the liver, spleen, kidney and bone marrow is stored mainly in the form of *ferritin*. Granick and Hahn (1944) showed that after intravenous injection of 9 mg. of radioactive iron (as ferric ammonium citrate) in dogs, 40 per cent was recovered in the liver after one hour, and 60 per cent after two hours. After 13 days 82 per cent of the iron was present in the liver; 75 per cent of the iron isolated from the ferritin rich fraction, and from the crystalline ferritin, was radioactive. Hahn *et al.* (1943) also showed that the iron of degenerating erythrocytes was similarly stored as ferritin in the liver. They transfused erythrocytes containing radioactive iron into dogs, to which they subsequently gave phenylhydrazine in order to produce rapid hæmolysis; after six days the liver contained 55 per cent of the radioactive iron, and some of this was present as ferritin.

Hahn (1948) has discussed the interesting possibility that *ferritin* may be identical with *hæmosiderin*. The latter pigment is found in large quantities in the macrophage-lymphoid system in diseases involving massive blood destruction, or following repeated blood transfusions, especially in patients with decreased hæmopoietic function, e.g. aplastic or pernicious anæmias; it is also found in large amounts in the tissues of patients with hæmochromatosis. Its presence is associated with the familiar Prussian blue reaction for the presence of iron in the tissues. It has been generally held that hæmosiderin is an iron-globin complex, and Hahn suggests that ferritin has a similar structure; it is of course true that apoferritin has a molecular weight of 460,000 whereas that of globin is only 68,000, but the former may be formed from the latter by aggregation. Both ferritin and hæmosiderin can be formed in the lymphoid-macrophage system under the same conditions and from similar starting materials. Hæmosiderin is furthermore available for hæmoglobin production in dogs with experimental anæmia, and Hahn suggests that hæmochromatosis might be treated by repeated bleeding to induce an anæmia.

Granick (1949) has reviewed the question of iron metabolism in *hæmochromatosis*. The fundamental abnormality in this disease

appears to be excessive absorption of iron from the gut, perhaps due to a greater reducing power of the mucosal cells which form more ferrous iron than usual. The serum iron level is raised.

It is interesting to note the differences in *availability for hæmoglobin formation* of the different forms of iron. Iron liberated by *hæmolysis* is immediately used again to re-form hæmoglobin, in preference to the available reserves (Cruz *et al.*, 1942), and *iron salts*, given by mouth or by injection, are probably as readily used for the same purpose (Dubach *et al.*, 1946). It has been suggested by Greenberg and Wintrobe (1946) that these two forms of iron represent a "labile iron pool" on which the first calls are made for hæmoglobin formation, and that the iron reserves are not drawn upon until the pool runs dry. There is, however, still much to be learnt about the factors concerned with the different forms of stored iron, the mechanisms of uptake and removal, and the maintenance of the serum iron level.

It is perhaps worth recalling the work of Brock and Hunter (1937), and Fowler and Barer (1937), who studied iron balances in patients with hypochromic anæmia who received large therapeutic doses of ferric ammonium citrate or ferrous carbonate by mouth. With the former compound, 30–35 per cent was retained in the body, but only 2 per cent was transformed into hæmoglobin. Thus large amounts of iron were stored in some form not available for hæmoglobin formation. It is not quite clear how this older work is to be correlated with recent investigations on radioactive iron, but the high percentage utilization of the latter (given by mouth) may be due partly to the fact that very much smaller doses have been given.

Excretion of Iron

As previously emphasized, iron excretion is very small and insignificant. Urinary excretion may amount to 0.5 mg. daily; biliary excretion (0.6–0.8 mg.) also occurs but some of this iron may be reabsorbed. In women menstrual blood loss may result in an iron loss of 10–40 mg. each month, or say an average of 1 mg. daily. Thus in men the average daily iron loss is about 1 mg. and in women about 2 mg. To cover these losses the diet for a man should contain about 5 mg. of iron, and women and growing children about 15 mg. At this level of intake the efficiency of absorption and utilization is about 10–12 per cent of the intake (Hahn, 1948).

However, our previous ideas on iron metabolism will have to be revised if the work of Mitchell and Hamilton (1949) is confirmed. They have found that sweat contains 1–2 mg. of iron/litre, a level which, under appropriate climatic conditions, could constitute as serious an iron loss as menstrual bleeding.

Hæmorrhage and Iron Deficiency Anæmia

After *severe blood loss during a short period of time*, the iron reserves of the body are adequate to re-form about 1/3 of the total hæmoglobin, though iron therapy will of course hasten recovery. With *chronic hæmorrhage* the iron reserves will be used up, and since *normal blood contains 50 mg. of iron/100 ml.* the iron loss from the body will be very serious. In these circumstances it would be very difficult to increase dietary iron absorption to as much as 20 mg. daily, and since up to 50 mg. daily may be required for maximal regeneration, iron therapy is essential for recovery. In many cases of iron deficiency anæmia, whether due to hæmorrhage or other causes, the total additional iron required to restore the normal hæmoglobin level is of the order of 1 G. or more; this is readily provided by administration of the usual iron salts, e.g. ferrous sulphate or ferric ammonium citrate. It may be useful to remember that *1 G. of hæmoglobin contains 3.34 mg. of iron*, and that about *25 mg. of iron are required to increase the hæmoglobin concentration by 1 per cent.* The doses required may be calculated from Table 11 from Witts (1936).

TABLE 11

Iron content and utilization of the average effective dose of various Therapeutic Preparations (*Witts*, 1936).

Preparation	Iron content of average dose	Per cent utilization
Ferrous chloride . .	100–200 mg.	12.5–25
Ferrous sulphate . .	180 mg.	14
Ferrous carbonate (Blaud's Pill) .	300–400 mg.	6–8
Ferric chloride . .	400 mg.	6
Ferric citrate . .	400 mg.	6
Iron and ammonium citrate	800–1,600 mg.	1.5–3

Infection and Iron Metabolism

It is very well recognized that infections and inflammatory states may be associated with anæmia. The mechanisms involved in the production of this anæmia have recently been studied by a number of workers. Hahn *et al.* (1946) produced turpentine abscesses in dogs and showed, by oral administration of radioactive iron, that *absorption* was reduced by this inflammation from the normal range of 18–41 per cent to 4–5 per cent of the total amount given. Wintrobe *et al.* (1947) showed in pigs infected with *Staphylococcus aureus* or *Escherichia coli*, that there was no increase in the rate of hæmoglobin destruction, but that *hæmoglobin production* was impaired. Greenberg *et al.* (1947) injected radioactive iron into normal rats and into rats in which abscesses had been produced by turpentine. They noted reduced hæmopoiesis, and increased deposition of iron in the liver and spleen ; there was no significant diversion of iron to the inflamed tissues. Greenberg *et al.* (1947) injected intravenous ferrous ascorbate into man, and showed that in the presence of infection the iron was lost from the plasma more rapidly than normal, and even when the plasma iron was raised to the levels which could be produced in normal subjects, hæmoglobin formation did not occur. Cartwright and Wintrobe (1949) measured the total iron-binding capacity of the serum in normal individuals and in patients with chronic infections. Their normal value was 359 μg. of iron/100 ml. serum. In infection, the values were mostly between 100 and 200 μg. of iron/100 ml. serum, and the percentage saturation of iron-binding protein had fallen from the normal level of 35 per cent to less than 20 per cent. Thus the anæmia of infection seems to be due to :—

(i) Reduced absorption of iron from the gut.
(ii) Diminished serum iron-binding capacity, and reduced serum iron level.
(iii) Diminished capacity for hæmoglobin synthesis.

Finch *et al.* (1949) have studied the utilization of *intravenously* injected radioactive iron in various human infections. In normal subjects 80 per cent of the injected dose of 1 mg. of ferric ammonium citrate was incorporated in hæmoglobin within 2–3 weeks. In patients with infections the figure was frequently reduced to less than 50 per cent, and in one patient with lobar pneumonia the utilization was only 14 per cent.

Intravenous Iron

The desirability of suitable iron preparations for parenteral administration has frequently been expressed, since some patients with hypochromic anæmia cannot be satisfactorily treated with the usual preparations given by mouth. A significant proportion of people suffer gastro-intestinal upsets with orally administered iron, and others fail to absorb iron given by this route. Heath *et al.* (1932) found that in man parenteral iron was about 100 per cent utilized for hæmoglobin formation, and that 32 mg. of ferric ammonium citrate by subcutaneous or intramuscular injection was equivalent to about 1,000 mg. by mouth. The difficulty has of course been the toxicity of the preparations used. Heath *et al.* (1932), for example, reported severe pain at the site of injection for 24 hours, and general reactions such as flushing of the skin, palpitations, and nausea, vomiting and diarrhœa, and central nervous depression. Intravenous injection of some preparations has produced embolic phenomena, presumably from precipitation of the iron compound, or from flocculation of plasma proteins, in the blood stream. After intravenous injection of iron preparations the iron in excess of that which can be bound by the plasma β_1 globulin, will rapidly leave the blood stream and become part of the labile iron pool. This unbound iron is probably responsible for the general reactions which follow intravenous injection of some iron preparations.

Attempts have been made in recent years to find new iron preparations for parenteral administration. For example, Ruskin and Katz (1936) tried ferrous adenylate, Little *et al.* (1945) gave ferrous ascorbate intravenously, Hahn (1946) introduced a colloidal ferrous iron preparation and Goetsch *et al.* (1946) tried colloidal ferric hydroxide by intravenous injection. These preparations have proved unsatisfactory, mainly because of their toxicity. An important advance in this field was made by Nissim (1947). He first found that intravenous administration of colloidal ferric hydroxide produced pyrexial reactions and local thrombophlebitis; he then tried a solution of *saccharated iron oxide* which was much better tolerated, and with doses up to 250 mg. of elemental iron practically no reactions were seen. Doses of 500 mg. and 1 G. did produce some reactions. Nissim noted that the iron was completely utilized in hæmoglobin synthesis.

Preparation and Standardization of Saccharated Iron Oxide for Intravenous Injection

Nissim and Robson (1949) have given a detailed account of the method of preparation of saccharated iron oxide for intravenous administration. The essential chemical reaction is the interaction of ferric hydroxide with sucrose in the presence of sodium hydroxide to produce iron sucrate (saccharated iron oxide). However, the reaction is very variable, depending largely on the properties of the ferric hydroxide, and some samples of iron sucrate may be only partially soluble in water. Nissim and Robson found that the best procedure is to add ferric chloride to sodium carbonate which react according to the equations :—

(i) $2FeCl_3 + 3Na_2CO_3 \longrightarrow 6NaCl + Fe_2(CO_3)_3$

(ii) $Fe_2(CO_3)_3 + 6H_2O \longrightarrow 2Fe(OH)_3 + 3H_2CO_3$

The most soluble iron sucrate is produced when the ferric hydroxide is dissolved in excess ferric chloride and then reprecipitated with sodium carbonate. The sodium chloride is then removed by washing with distilled water, and after decanting, sucrose and sodium hydroxide are added (pH about 11.7). The mixture is then heated in a flat porcelain dish in an oven at 130°C. and the saccharated hydroxide gradually dissolves to an almost black solution. This is evaporated to dryness and the iron sucrate dissolved in distilled water to give a solution containing 2 per cent elemental iron, which is filtered and sterilized by autoclaving at 115–116°C. for half an hour. The original paper must be consulted for details of this method of preparation, and for a discussion of the many variables which may influence the nature of the final product.

Nissim and Robson (1949) have shown that saccharated iron oxide preparations must be standardized biologically by toxicity tests in mice. A sample which had been successfully used clinically was chosen as the standard ; its LD50 on intravenous injection was 180 mg. of iron per kg. body weight. The best sample prepared by Nissim and Robson had an LD50 of 300 mg./kg. in mice, and up to 300 mg. of such samples were given intravenously in man with no toxic effects. Studies of the toxicity of saccharated iron oxide in mice revealed no evidence of any cause of death apart from precipitation of iron in the lungs, intestine, liver, kidney and heart.

The dark colour of saccharated iron oxide solutions makes it impossible to test the intravenous location of the needle tip by the usual procedure of withdrawing blood by suction with the piston of the syringe. On the other hand the colour soon reveals the presence of peri-venously injected solution.

Effects of Intravenous Saccharated Iron Oxide in Anæmia

Nissim's first report was mainly concerned with the demonstration that iron-sucrose solutions were well tolerated on intravenous injection, but he recorded large increases in hæmoglobin levels in two patients after infusions of 500 mg. of iron. Nissim and Robson (1949) have reported dramatic responses after 300 mg. of iron in their new preparation in patients with hypochromic anæmia who were refractory to oral iron. Slack and Wilkinson (1949) have given intravenous iron-sucrose to 60 patients with iron-deficiency anæmia (10 refractory to oral iron) and reported very striking clinical and hæmatological responses. They usually gave initial doses of 25 mg. of iron daily, and then gradually increased these up to 200 mg. daily or twice daily, and noted practically no local or general reactions (300 mg. of iron in their preparation did produce some reactions). They confirmed previous findings that injected iron is nearly 100 per cent utilized for hæmoglobin formation, but patients with chronic infection required much larger doses than the others to raise the hæmoglobin level to normal. Govan and Scott (1949) administered the preparation Ferrivenin to 25 anæmic pregnant women, and recorded rapid increases in hæmoglobin concentration, e.g. 8 per cent in one week; oral iron is too slow in action, is frequently not tolerated, and is sometimes ineffective in such cases. Sinclair and Duthie (1949) have described the results of intravenous iron therapy in patients with hypochromic anæmia associated with rheumatoid arthritis, who did not respond to iron by mouth. They noted good responses to Ferrivenin in those patients who had a low erythrocyte sedimentation rate, but observed very little improvement in those who had a high E.S.R.

Since intravenous iron is quantitatively converted to hæmoglobin it should be possible to calculate the amount of iron required to raise the hæmoglobin concentration to normal; 100 mg. of elemental iron should increase the blood hæmoglobin

by 0.55 G./100 ml. (4 per cent on the Haldane scale), but in chronic iron-deficiency anæmia the iron reserves are depleted to an unknown degree. Slack and Wilkinson (1949) suggest that 50 per cent should be added to the calculated iron requirement in order to raise the reserve iron to normal.

The *toxic effects* which result from the administration of unduly

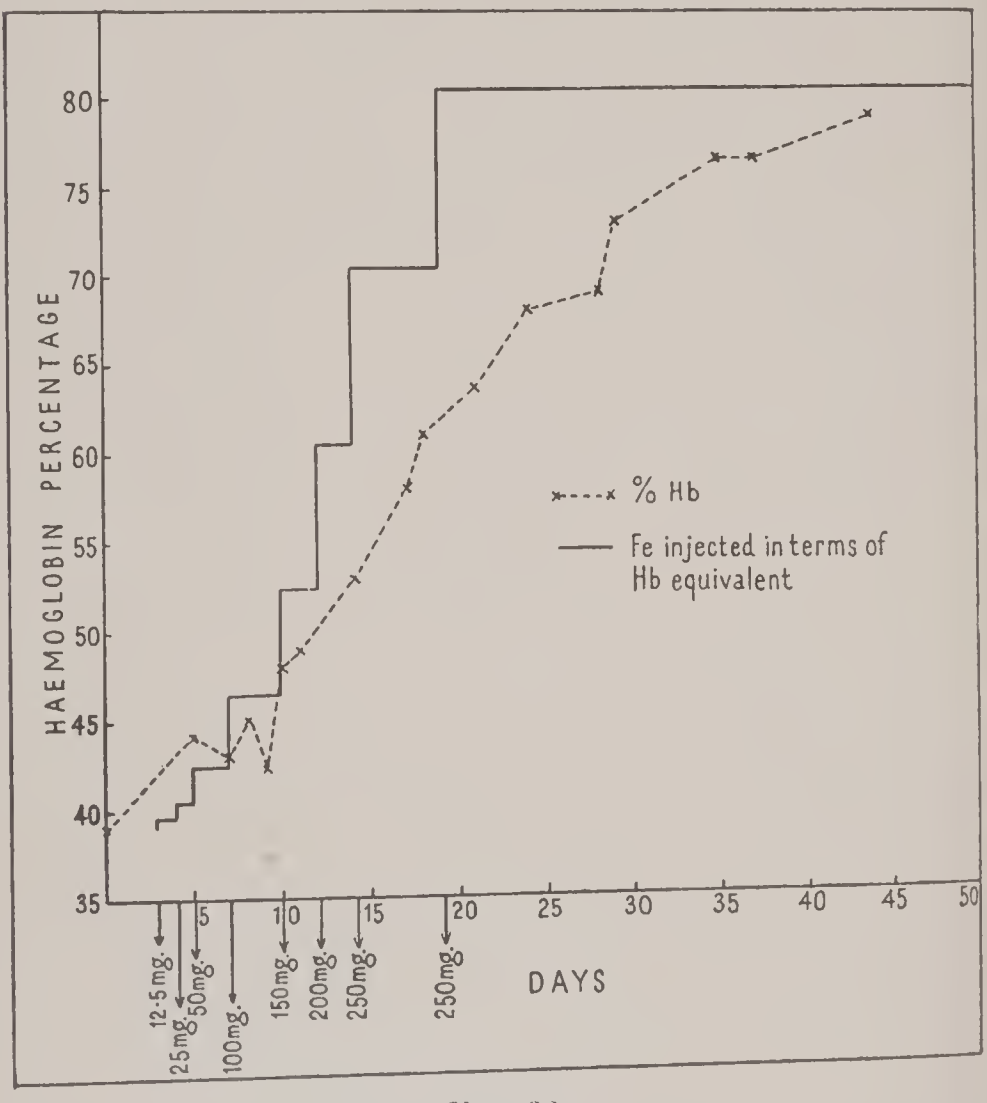


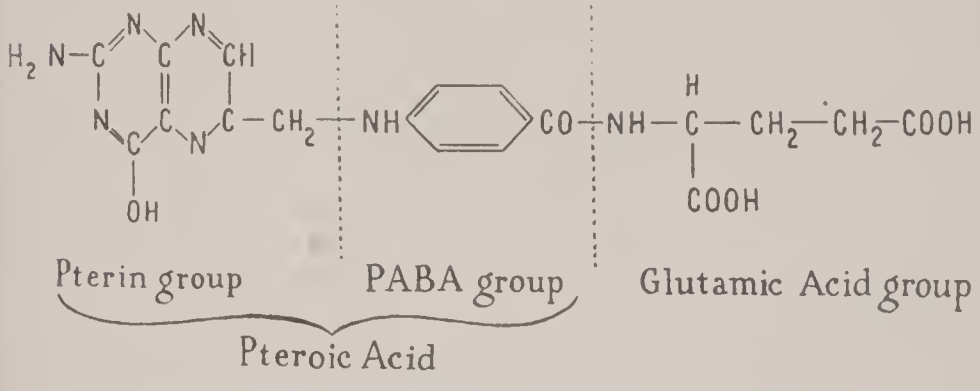
FIG. 32

Showing the effect of intravenous injections of saccharated iron oxide on hæmoglobin formation. The patient was a girl of 19 with hypochromic anæmia who failed to improve on oral iron. The graph shows the actual and theoretical responses to the injections. The patient maintained her improvement for 8 months (after which she no longer attended hospital).

large doses of saccharated iron oxide are those of multiple embolism in the pulmonary and systemic circulation. They include rigors, collapse with fall in blood pressure, tachycardia, dyspnœa and cyanosis, pleuritic and low back pain, abdominal pain with diarrhœa, etc. Subsequently pyrexia may develop. The threshold at which such symptoms occur depends on the actual preparation used. With Ferrivenin, and with Slack and Wilkinson's preparation, the upper safe limit seems to be 200 mg. Fe per injection (Slack and Wilkinson, 1949). With the best samples reported by Nissim and Robson (1949) doses of 300 mg. Fe appear to be completely free from reactions.

With certain samples, impurities in the sugar of saccharated iron oxide appear to be responsible for a toxic reaction of a different category characterized chiefly by rapid onset, transient duration and freedom from sequelæ. This reaction is, however, very unpleasant to the patient who experiences severe pain in the back and in the chest and arms which is similar in character and distribution to the pain of angina. The best samples of saccharated iron oxide prepared with Analar sucrose appear to be free from such reactions.

Pteroylglutamic Acid (Folic Acid)



Pteroylglutamic Acid (Folic Acid)

Pteroylglutamic acid is a recently identified constituent of the vitamin B group, which occurs naturally, chiefly as conjugates, in such food sources as yeast, liver, kidney, milk, grasses and green leaves. Its isolation by Mitchell *et al.* (1941) from spinach and other green leaves led to the name "Folic Acid," but the term

pteroylglutamic acid (PGA) is more satisfactory as it is chemically descriptive. Folic acid antagonists are discussed in Chap. 15.

Conjugates of PGA

Pteroyltriglutamic acid (PTGA) and *Pteroylheptaglutamic acid* (PHGA) are formed by the attachment in peptide linkage of 2 and 6 glutamic acid residues respectively to the glutamic acid radical of PGA.

Jukes and Stokstad (1948) have reviewed the many convergent lines of experimental work in this field. Firstly, it has been shown that PGA is essential for the growth of certain bacteria, e.g. *Streptococcus lactis R.* and *Lactobacillus casei* : secondly, it has been found to be necessary for certain actions in different species of animals. For example, it is almost certainly identical with vitamin M, which is effective against a macrocytic hyperchromic anæmia with leucopenia and granulocytopenia, produced experimentally in rhesus monkeys, and also with vitamin B_c, which prevents nutritional anæmia in chicks. In rats, PGA is apparently formed in adequate quantities by bacterial action in the intestine, and sterilization of the alimentary tract by administration of succinylsulphathiazole or sulphaguanidine leads to leucopenia, agranulocytosis, aplastic anæmia and thrombocytopenia, all of which are corrected by administration of PGA.

It is of interest that p-aminobenzoic acid functions as a precursor of PGA in the nutrition of certain bacteria, and that the bacteriostatic action of the sulphonamides in such organisms may be due to inhibition of the enzyme system which synthesizes PGA from p-aminobenzoic acid (see Chap. 15).

It has in addition been found that *thymine* is an effective substitute for PGA in promoting growth of *Lactobacillus casei* and *Streptococcus lactis R.*

Pteroylglutamic Acid in Pernicious Anæmia

The actions of synthetic PGA in pernicious anæmia were reported first in America by Vilter *et al.* (1945) and by Moore *et al.* (1945). The position has been reviewed by Davidson (1948) and by Wilkinson (1948), who summarize the facts as follows :—

(1) PGA, whether given by mouth (usually 10–20 mg. daily) or as the disodium phosphate salt parenterally, produces a prompt reticulocytosis within 5–10 days, but it is generally agreed that the

peak response is less than with potent liver extracts (Haden, 1948 ; Clark, 1948).

(2) The megaloblastic sternal bone marrow is rapidly converted to a normoblastic one, this change always being complete before the maximal reticulocyte response occurs.

(3) The increases in blood hæmoglobin concentration and red cell count are comparable in rate and completeness to those which follow the administration of liver extract (Fig. 33), but subsequently the values may not be so well maintained, and slight

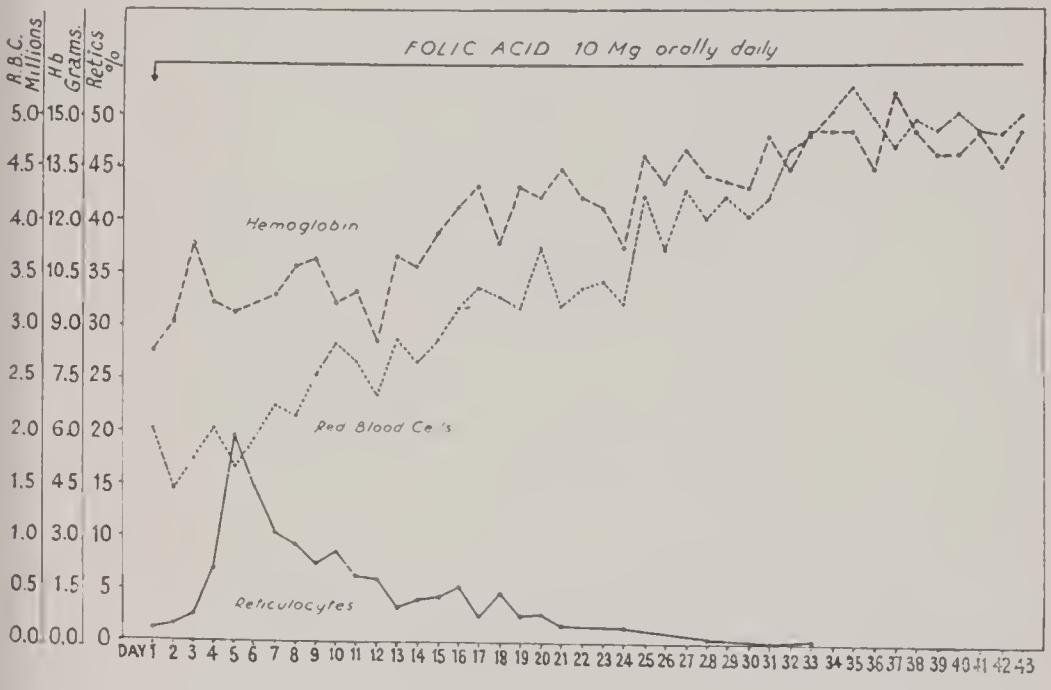

FOLIC ACID 10 Mg orally daily
R.B.C. Millions
Hb Grams.
Retics %
Hemoglobin
Red Blood Cells
Reticulocytes
DAY 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43

FIG. 33

Response of patient with pernicious anæmia to folic acid. (After Spies, 1947.)

macrocytosis may be observed. White cell and platelet counts also increase.

(4) Clinical symptoms improve rapidly after PGA in much the same way, and at the same rate, as with liver extract or hog's stomach preparations. The patient feels better even before the reticulocyte peak occurs, and improvement in alimentary symptoms soon follows, though glossitis may be less affected than with liver therapy (Ross *et al.*, 1948). Central nervous symptoms, however, persist.

PGA and Neurological Complications of Pernicious Anæmia

A most important aspect of the action of PGA in pernicious anæmia, from both the theoretical and practical viewpoints, is the absence of effect on the neurological complications of this disease. It has become abundantly clear that peripheral neuritis and subacute combined degeneration of the spinal cord are not improved by PGA, and further, that these complications may actually arise in patients being treated with this substance, even after a good hæmatological response has been produced (Spies and Stone, 1947). Wilkinson and Israels (1947) added figures recorded from the literature to their own, and showed that of 180 patients with pernicious anæmia, treated with PGA, 60 developed severe neurological complications, which subsequently improved after treatment with liver extract or hog's stomach. It has been suggested that PGA might produce damage to the central nervous system, but this seems most unlikely since such involvement does not occur when other types of macrocytic anæmia are treated with this substance.

These findings make it clear that PGA should never be used alone in the treatment of pernicious anæmia, though Dameshek (1948) thinks it may be of value as an adjuvant to liver therapy.

Other Considerations About PGA in Pernicious Anæmia

(1) PGA is not the extrinsic factor of Castle since it is active without pre-digestion with gastric juice, and is effective on injection.

(2) PGA is not hæmopoietin (Castle's intrinsic factor) which is readily destroyed by heat.

(3) PGA is clearly not the active principle present in liver extracts since the effective daily dose of PGA is 10–20 mg. while the daily requirement of a potent liver extract contains only 0.02–3.7 μg. of PGA (Clark, 1945).

An interesting point concerning the mode of action of liver extract has been raised by the work of Heinle *et al.* (1947) and Bethell *et al.* (1947) on conjugated PGA. They found that in normal persons parenteral administration of pteroylheptaglutamic acid (PHGA) produced a considerable excretion of PGA in the urine, whereas in patients with pernicious anæmia there was no such response. However in three patients with pernicious anæmia,

who were well controlled by liver therapy, PHGA administration produced the same increase in urinary excretion of PGA as seen in normal subjects. These authors suggested that the anti-anæmic liver principle might act as a conjugase, splitting PHGA to form PGA, which could then act on the bone marrow to stimulate normal erythropoiesis. This obviously cannot be the whole story, but it is interesting to note that PGA is present in foods containing the vitamin B complex in the conjugated form (PHGA).

It should, however, be added that Wilkinson and Israels (1949) recorded different results when they administered two synthetic folic acid conjugates, pteroyl-γ-triglutamic acid (teropterin) and pteroyl-α-diglutamic acid (diopterin) to patients with pernicious anæmia. When given by intramuscular or intravenous injection in doses of 40-100 mg. daily, these substances produced the same kind of response as occurs with PGA alone. These authors, therefore, suggest that folic acid conjugates are split normally by patients with pernicious anæmia, and they consider that previous failures to demonstrate such an action were due to the use of too small doses of conjugates, and to the presence of conjugase inhibitors in the concentrates employed.

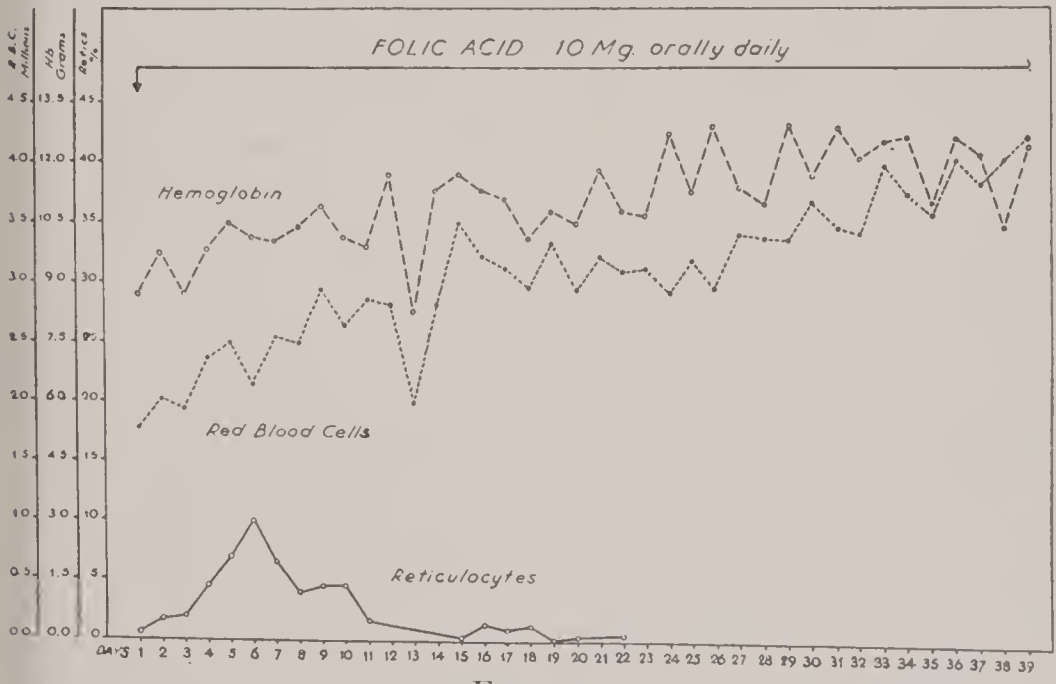


FIG. 34

Response of patient with nutritional macrocytic anæmia to folic acid. (After Spies, 1947.)

PGA in Other Anæmias

It may be stated generally that PGA is effective only in the treatment of macrocytic anæmias with some megaloblastic change in the bone marrow. These conditions include :—

(*i*) *Nutritional Macrocytic Anæmia.* Spies *et al.* (1948) observed satisfactory clinical and hæmopoietic responses with PGA in 32 patients with this type of anæmia (see Fig. 34).

(*ii*) *Liver-refractory Macrocytic Anæmias.* Davidson (1948) has reported excellent therapeutic effects with PGA in 25 cases of idiopathic liver-refractory megaloblastic anæmia ; proteolysed liver was also effective, partly owing to its content of PGA.

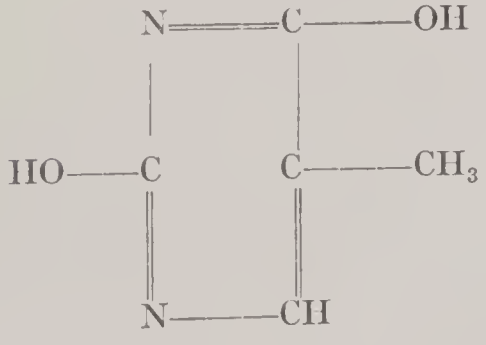
(*iii*) *Sprue.* In *tropical sprue* Lopez *et al.* (1946) reported excellent hæmatological responses with PGA, but it is probable that their patients were also suffering from a direct nutritional deficiency leading to a megaloblastic bone-marrow. Of ten patients with the sprue syndrome seen in Edinburgh by Davidson *et al.* (1947) only one showed a hæmatological response to PGA, and this case was also the only one with a megaloblastic bone-marrow.

It is interesting that in tropical sprue and in idiopathic steatorrhœa, PGA controlled diarrhœa and produced clinical improvement, whatever the hæmatological effect. PGA did not improve fat absorption (Davidson *et al.*, 1947).

Blood Diseases Which Do Not Respond to PGA

PGA has no beneficial effects in hypochromic microcytic anæmias, hæmolytic anæmias, refractory megalocytic anæmias with normoblastic marrows, acute and chronic leukæmias of all types, aplastic or hypoplastic anæmias, agranulocytosis, neutropenias or thrombocytopenic purpura (Wilkinson, 1948).

Thymine



Thymine (5-methyl uracil)

Spies *et al.* (1946) found that thymine in daily dosage of 4.5–10 G. produced a reticulocytosis and increases in hæmoglobin and red cell count in three patients with pernicious anæmia, and Lopez *et al.* (1948) described its effectiveness in tropical sprue.

Like PGA it had no effect on the neurological complications of pernicious anæmia (Spies and Stone, 1947).

Comparisons of Thymine, PGA, and Vitamin B_{12}

Spies *et al.* (1948) studied the comparative effectiveness of thymine, PGA and vitamin B_{12} in nutritional macrocytic anæmia, tropical sprue and pernicious anæmia. They established the following approximately equivalent doses for these compounds :—

Thymine 6 — 10 G. orally
≡ 10 mg. PGA orally ≡ 6 — 23 μg. vitamin B_{12} intramuscularly.

Vitamin B_{12}

During the past twenty years much work has been done in the search for the liver substance active in pernicious anæmia, and recently a most important advance has been made (see review by Smith; 1949). Rickes *et al.* (1948) in America, and Lester Smith (1948) in this country, almost simultaneously announced the isolation from liver of an extremely potent red pigment, which the former workers were the first to prepare as small crystalline red needles. In their study of the activity of various liver fractions Rickes *et al.* (1948) used a test devised by Shorb (1948), who found that the micro-organism *Lactobacillus lactis* Dorner requires for its growth a substance apparently identical with the anti-anæmic substance of liver. The red crystalline material, which was named "*vitamin* B_{12}," was many thousands of times as active as a standard liver concentrate in promoting the growth of the lactobacillus, and preliminary clinical studies by West (1948) showed that as little as 3–6 μg. of the substance produced reticulocytosis in patients with pernicious anæmia. Rickes *et al.* (1948) stated that 1 μg. of vitamin B_{12} was equivalent in activity to 1 U.S.P. injectable unit (which is the daily amount of liver extract required to produce a satisfactory clinical and hæmatological response) and suggested that 20–60 μg. of vitamin B_{12} would probably be required to produce a remission in pernicious anæmia.

Studies on the *chemistry* of vitamin B_{12} have been reported by Lester Smith (1948) and by Rickes *et al.* (1948). The first interesting finding was the demonstration of the presence in the crystals of 4 per cent cobalt, which is probably responsible for the red colour. If each molecule of vitamin B_{12} contains 1 atom of cobalt the molecular weight is about 1,500. Rickes *et al.* (1948), from emission spectrographic analysis, have shown that there are six groups about the cobalt atom. Other constituents of the molecule include glutamic acid, and three atoms of phosphorus. Holliday and Petrow (1949) have obtained evidence which suggests that vitamin B_{12} may be regarded as a 5 : 6-dimethylbenziminazole derivative, and Ellis *et al.* (1949) suggest that vitamin B_{12} also contains 2-aminopropanol.

The rôle of *cobalt* in erythropoiesis is difficult to evaluate (see review by Cartwright, 1947). In cattle and sheep a deficiency of cobalt in the diet may produce a normocytic hypochromic anæmia, and in a number of other animal species addition of small amounts of cobalt to the diet produces a true polycythæmia with hyperplasia of the bone marrow, which may be counteracted by administration of whole liver or liver extract. Large amounts of cobalt depress erythropoiesis. It is not known what is the requirement for cobalt in man (the diet contains about 100 μg. of cobalt daily) and this metal has not been found effective in the treatment of anæmia. Rickes *et al.* (1948) found that the cobaltous ion in relatively high concentrations had no effect on the growth of *Lactobacillus lactis* Dorner, and West and Reisner (1949) found that 150 μg. of cobaltous chloride daily was ineffective in a patient with pernicious anæmia. Robinson *et al.* (1949) observed a small increase in erythropoiesis after administration of cobalt to patients with anæmia due to chronic sepsis or neoplasms, but they obtained no response in patients with pernicious anæmia, even when cobaltous chloride was previously incubated with normal gastric juice. The significance of the presence of cobalt in vitamin B_{12} remains to be assessed.

Production of Vitamin B_{12} by Streptomyces Griseus

Rickes *et al.* (1948) have recorded the most interesting finding that *Streptomyces griseus*, the organism which produces streptomycin, also produces vitamin B_{12}, which chemical, physical and biological tests showed to be identical with the vitamin B_{12}

isolated from liver concentrates; its clinical effectiveness in pernicious anæmia has also been established (Erf and Wimer, 1949; Dunlop and Wilson, 1949). It is thus possible that in the future, treatment of patients with pernicious anæmia will employ anti-anæmic substance from this source instead of liver, and that the activity of the material will be biologically standardized by its influence on the growth of *Lactobacillus lactis* Dorner. It would certainly be most satisfactory to find some alternative and more regular source of anti-anæmic principle than liver, but much work obviously remains to be done, not only in the commercial production of the substance by *Streptomyces griseus*, but also in the correlation of the growth-promoting influence of vitamin B_{12} in *Lactobacillus lactis* Dorner with the clinical response in pernicious anæmia. It should, however, be mentioned that Cuthbertson and Smith (1949) have shown that three other microbiologically active substances (besides vitamin B_{12}) can be isolated from liver extracts by partition chromatography on paper, so the final identification of the anti-pernicious anæmia factor by the microbiological method is not yet certain. Moreover, there is considerable variation in the *L. lactis* Dorner activity among different brands, and even among different batches of the same brand, of liver extracts (Shaw, 1949). The presumptive vitamin B_{12} content of "high potency" liver extracts is of the order of 10 μg./ml. Clinical responses to extracts containing less than 10 μg./ml. of vitamin B_{12} were much less satisfactory than to extracts containing more than 10 μg./ml. (Cuthbertson *et al.*, 1949). One thing is quite clear, at present it would be quite impractical to prepare vitamin B_{12} from liver for therapeutic use, since four tons of liver yield only 1 G. of the vitamin.

Biological Actions of Vitamin B_{12}

Some preliminary reports have shown that vitamin B_{12} stimulates growth in young animals, e.g. rats and pigs (Emerson *et al.*, 1949; Hogan and Anderson, 1949). It also acts like liver extract to antagonize the thyrotoxicosis which occurs in chicks when they are fed with iodinated casein (Nichol *et al.*, 1949).

Action of Vitamin B_{12} in Pernicious Anæmia

Hæmatological Response. So far there have been a number of what must be regarded as preliminary reports on the actions of

vitamin B_{12} in pernicious anæmia (see review by Reisner, 1949). It will clearly take some time yet to make a final assessment of its long term effectiveness, but there is no doubt whatever of its capacity to induce a characteristic clinical and hæmatological remission in this disease. The original report by West (1948) has been followed by numerous demonstrations of the efficacy of crystalline vitamin B_{12} in pernicious anæmia (e.g. Hall and Campbell,

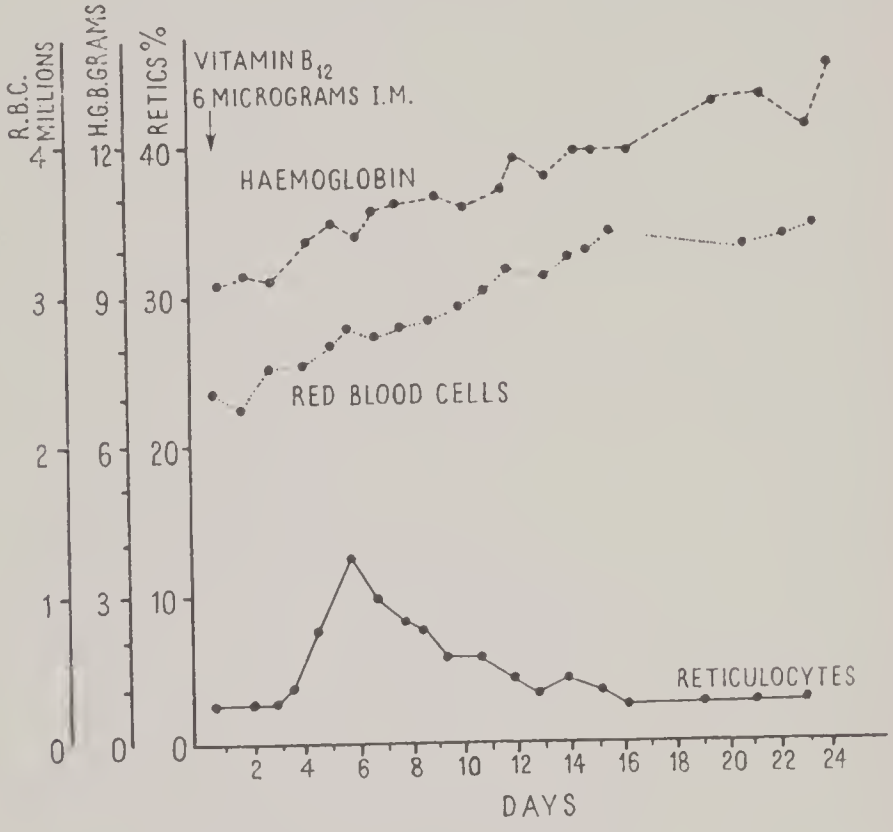


Fig. 35

Action of Vitamin B_{12} in a Patient with Pernicious Anæmia. (After Spies *et al.*, 1949.)

1948; Ungley, 1948 and 1949; Berk *et al.*, 1948; Spies *et al.*, 1949; West and Reisner, 1949; Mettier *et al.*, 1949; Jones *et al.*, 1949). The early responses to administration of vitamin B_{12} are identical with those which follow liver therapy. Within a few days the patient feels stronger, becomes mentally more alert, his appetite returns and a gain in weight soon results; the signs and symptoms of glossitis are alleviated within 4–5 days. The hæmatological response is very striking; Hall and Campbell (1948)

record that after injection of 25 μg. of vitamin B_{12} the megaloblastic bone marrow picture was changed to a normoblastic one within 48 hours, and indeed in one patient there was a significant alteration of the marrow count within nine hours of injection of 1 μg. of vitamin B_{12}. Reticulocytosis, in patients with initial red cell counts below two million per c.mm., may reach as high as 40 per cent within 4–7 days of injection, and subsequent increases in hæmoglobin concentration and red cell count are similar to those which follow liver therapy.

The effects of vitamin B_{12} on *neurological complications* are of great importance (remembering the early high promise of folic acid), and it may already be said that vitamin B_{12} has beneficial effects on peripheral neuritis and on subacute combined degeneration of the cord associated with pernicious anæmia. Spies *et al.* (1949) have reported that in one patient, whose symptoms from combined degeneration were steadily progressing during folic acid administration, injection of vitamin B_{12} produced dramatic subjective and objective improvement within four days. Other reports (e.g. Hall and Campbell, 1948; Berk *et al.*, 1948; Mettier *et al.*, 1949; Ungley, 1949) support the view that vitamin B_{12} acts like liver extract on these neurological complications, but it is clearly too early yet to assess the final results.

Berk *et al.* (1948) found that in a patient who showed *allergic hypersensitivity* to pork and beef liver extracts, there was no reaction to injections of vitamin B_{12}. This is obviously a matter of practical importance.

Vitamin B_{12} and Extrinsic Factor (Castle)

Berk, Castle *et al.* (1948) have shown that the hæmopoietic activity of orally administered vitamin B_{12} in pernicious anæmia is enhanced by simultaneous administration of normal gastric juice, but that such activity is still below that of injected vitamin B_{12}. They suggest that the extrinsic factor in food (e.g. beef muscle, milk and eggs) may be identical with or closely related chemically to the anti-pernicious anæmia principle of liver, which in turn is presumed to be identical with vitamin B_{12}. Berk *et al.* have further stated that, "It is therefore possible that the function of the intrinsic factor of normal human gastric juice is to facilitate the absorption by the intestine of vitamin B_{12} or of chemically

related compounds in the food, rather than to react with the extrinsic factor as hitherto assumed."

It has been shown by Bethell *et al.* (1948) that the daily output of vitamin B_{12} in the fæces of patients with untreated pernicious anæmia is much greater than that necessary to produce remission when given by injection, and they suggest that this disease may be due to defective absorption of vitamin B_{12}, whether derived from the diet or from intestinal bacterial synthesis.

Folic Acid and Vitamin B_{12}

Bethell *et al.* (1948) reported that the folic acid antagonist, aminopterin, inhibited the response to injected vitamin B_{12} in a patient with pernicious anæmia. It has since been shown by Meyer *et al.* (1949) that other folic acid antagonists, pteroyl aspartic acid (An-Fol-R) and methyl pteroic acid (Met. Fol-B) prevented the responses of patients with pernicious anæmia to injections of liver extract or of vitamin B_{12}. These findings naturally raise the question of synergistic actions between folic acid and vitamin B_{12}, but it is too early yet to discuss the possible mechanisms involved.

Actions of Vitamin B_{12} in Other Anæmias. Patel (1948) and Spies *et al.* (1949) have reported good responses to the administration of vitamin B_{12} in *tropical macrocytic anæmia*, *nutritional macrocytic anæmia* and in *sprue*. Spies *et al.* (1949) found that vitamin B_{12} was ineffective in secondary anæmia, aplastic anæmia, leukopænia following infections or due to drugs, and in leukæmia.

Dosage and Administration

So far it has been found necessary to give vitamin B_{12} by intramuscular injection; Hall and Campbell (1948) found oral administration to be ineffective, though Spies *et al.* (1949) did observe slow responses when vitamin B_{12} was given by mouth in doses 30–60 times greater than those required by injection. The dosage used has varied greatly. Hall and Campbell (1948) obtained responses in two patients with pernicious anæmia with 1 μg. daily, but some cases may not respond to an injection of 10 μg. and Spies *et al.* suggest that the average patient responds maximally to 100 μg. Erf and Wimer (1949) noted that in previously untreated patients 50–100 μg. of vitamin B_{12} produced remissions lasting 50–100 days, and that in patients already in remission, 50 μg. maintained an effect for 70–120 days.

In his extensive, well controlled studies on 53 patients with pernicious anæmia, Ungley (1949) has recorded the responses to initial doses of vitamin B_{12} ranging from 1.25 to 160 μg. He found that, as judged from the reticulocyte response or by the rise in the total red cell count during 15 days, a single dose of 10 μg. of vitamin B_{12} was as effective as the usual liver extracts, and that in most patients an injection of 10 μg. every two weeks was a satisfactory maintenance dose. Ungley suggests that, to take account of individual variations in response, uncomplicated cases should be given 40–80 μg. weekly for the first three months and 30 μg. every three weeks thereafter. Patients with subacute combined degeneration should receive larger and more frequent doses.

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CHAPTER 14

ANTICOAGULANT DRUGS

In recent years there has been increasing recognition of the value of anticoagulants in the therapeutic control of thrombophlebitis and related conditions. Two substances, Heparin and Dicoumarol, have been used for this purpose.

Heparin

A full discussion of the chemistry, physiology and medical applications of heparin has been presented by Jorpes of Stockholm in his book "Heparin in the treatment of thrombosis" (1946), and a general account of "Anticoagulants effective *in vivo*" (including heparin) has been given by Quick (1944).

Heparin was discovered by Howell and his collaborators in 1916, and it was so named because of its predominant localization in the liver. Howell discovered methods for its preparation and suggested that it was a physiological anticoagulant, responsible for the fluidity of the circulating blood. Subsequent work by Best, Charles and Scott in Toronto with improved extraction methods showed that heparin was present in tissues throughout the body, especially the lungs, thus emphasizing its possible physiological significance. In 1935–36 Jorpes found that heparin was chemically a polysulphuric ester of mucoitin.

Chemical Composition of Heparin

The chemical formula of heparin is not exactly known but it is a large polysaccharide molecule (M.W. about 700 or some multiple of 700) built up of disaccharide units which are the same as those found in mucoitin sulphuric acid. Each disaccharide unit consists of N-acetylated glucosamine linked with glucuronic acid in such a way that each unit probably has 4 OH groups available for combination with sulphate groups. Maximum anticoagulant activity occurs with the trisulphuric acid compound. Heparin preparations are not homogeneous even in the case of what has been called "crystalline heparin." Depolymerization and deacetylation completely abolish biological activity.

Source and Preparation of Heparin for Therapeutic Use

Heparin is a greyish brown powder. It is prepared from ox lung, and is made up in aqueous solution as the sodium salt. The B.P. Injection of heparin is a solution of heparin in physiological saline, containing not less than 90 per cent, and not more than 110 per cent of the number of units of heparin per ml. stated on the label. The pH of this solution is 7.0 to 8.5. The solution is sterilized by filtration, and when kept at a temperature not above 20°C. it shows no significant reduction in potency during two years. Heparin is expensive to produce for therapeutic use.

International Standard and Unit of Heparin

The Standard Preparation is a quantity of the dried sodium salt of heparin. The Unit is the specific activity contained in 0.0077 mg. of the Standard Preparation in use in 1947 (British Pharmacopœia, 1948, p. 807). 1 mg. of the Standard Preparation thus contains 130 units of activity. If the activity is expressed in terms of the barium salt 1 mg. contains 100 units, which is the figure given for commercial preparations of heparin. These latter contain 10 mg. (1,000 U) or 50 mg. (5.000 U) per ml. of solution. Jorpes suggests that the dosage should always be expressed in mg.

The British Pharmacopœia (1948) recommends the following blood or plasma preparations for heparin assay :—

(*a*) the freshly shed blood of the cat or other suitable animal,

(*b*) fowl plasma to which a tissue extract has been added to promote clotting, or

(*c*) the decalcified blood or plasma of various mammalian species used in conjunction with a solution of calcium salt alone, or combined with a tissue extract, or a solution of thrombin.

Mode of Action of Heparin

Heparin inhibits blood coagulation both *in vitro* and *in vivo*. It appears to act partly by preventing the conversion of prothrombin to thrombin, and partly by antagonizing the action of thrombin itself; in both these actions co-factors, present in the plasma, are necessary (Quick, 1944). Like all anticoagulants, heparin inhibits the clumping of blood platelets, an action which contributes to its capacity to prevent thrombosis *in vivo*

(Wright, 1945). Heparin has no chemically reactive groups in its molecule and its anti-coagulant actions are attributed to its physico-chemical properties, particularly to its exceptionally strong electronegative charge, due to the sulphuric acid groups. Other polysaccharide polysulphuric acid esters, both natural and synthetic, have marked anticoagulant properties for probably the same reason. Basic substances, such as toluidine blue and protamine, neutralize the negative electric charge of heparin and completely antagonize its anticoagulant action.

Administration and Duration of Action of Heparin

Heparin is usually administered *intravenously*, either continuously in a saline drip or, more frequently, by repeated injections. The duration of action after a single injection is brief, e.g. after 100 mg. (10,000 units) the coagulation time is increased for a period of about three hours (Fig. 36), the maximum effect occurring 5–10 minutes after injection (Takats, 1943). If the intravenous route is used, injections must be given at intervals of 4–6 hours to maintain a coagulation time exceeding 15 minutes (normal 4–7 minutes).

Attempts have been made to prolong the effects of heparin by using other routes of administration. Walker (1945) has tried intramuscular injections; subcutaneous injection was too painful and produced local reactions. A long-acting preparation for subcutaneous injection, was introduced by Loewe and Rosenblatt (1944), who incorporated heparin (100 mg./ml.) in Pitkin's Menstrum (18 per cent gelatin, 8 per cent glucose, 1 per cent acetic acid and distilled water). With this mixture, which has been used to prolong the action of other water soluble drugs, the onset of heparin action occurred at two hours and the maximum effect at 16 hours after injection, the total duration being some 40 hours (Evans and Boller, 1946). Another mixture used to prolong heparin action has been described by Vorzimer *et al.* (1948) who made an emulsion of an aqueous solution of heparin (200–300 mg./ml.) in a menstruum consisting of: cholesterol derivatives 33 per cent, peanut oil 65 per cent and beeswax 2 per cent. A single intramuscular injection prolonged the clotting time adequately for up to 24 hours. The dosage of heparin will be discussed later.

Distribution and Fate of Heparin

Heparin is a relatively large molecule which becomes attached to plasma proteins ; it therefore remains in the blood stream and does not diffuse through natural or artificial membranes (e.g. the cellophane of the artificial kidney).

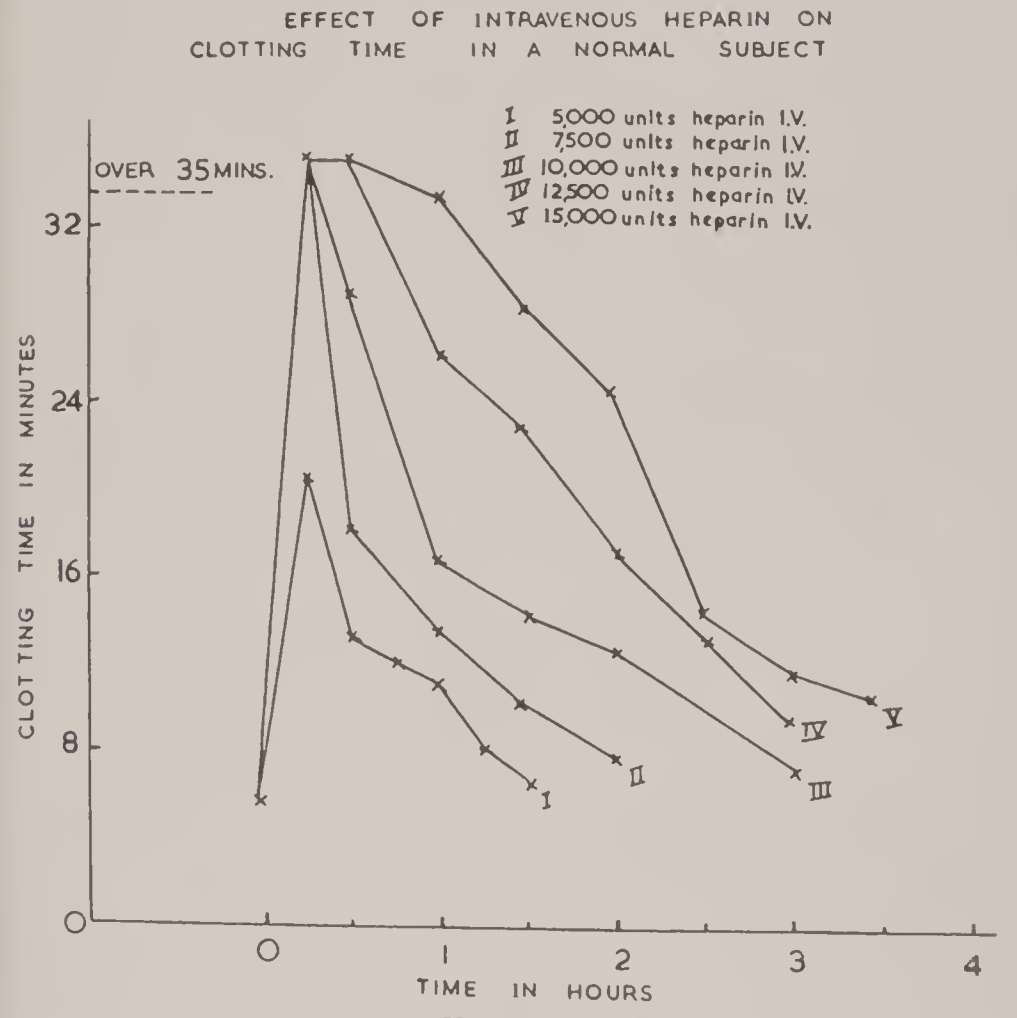


Fig. 36

Heparin and Clotting Time

Effects of different doses of heparin given intravenously. Note the brief duration of action.

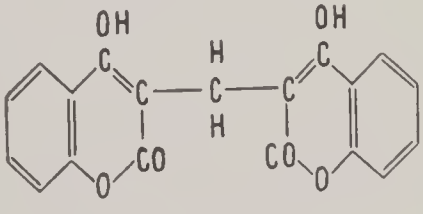
Ordinate. Clotting time in minutes. Abscissa. Time in hours. The clotting time was determined by a new capillary tube method devised by Dr. J. W. Stewart of the Bland-Sutton Institute of Pathology, Middlesex Hospital. The normal clotting time by this method is 4–8 minutes. These records were obtained by Mr. L. P. LeQuesne of the Surgical Unit, Middlesex Hospital.

The fate of injected heparin and the reasons for its brief duration of action are unknown. It was formerly stated that heparin was partly excreted in the urine, but Jaques and Ricker (1948) could find no evidence of this, and showed that nephrectomy in dogs did not alter the duration of action of heparin. There is no evidence that the liver is concerned with the disappearance of heparin, and none appears in the fæces.

Variations in Sensitivity to Heparin. Some difficulty in the use of heparin arises from individual variations in response. Takats (1943) devised a "heparin tolerance test" in which 10 mg. of heparin was injected intravenously and the effect on coagulation time was recorded before and 10, 20, 30 and 40 minutes after injection. He classified subjects as normal, hypo- and hyper-reactors according to the maximum recorded prolongation of clotting time.

Dicoumarol

Dicoumarol was discovered in a most interesting way. Schofield (1922) described a hæmorrhagic disease in cattle which was thought to be due to the eating of spoiled hay from sweet clover, and subsequently Roderick (1931) found that in this disease the plasma prothrombin level was reduced in parallel with a prolongation of coagulation time. Quick (1937) produced "sweet clover disease" experimentally in rabbits and showed that the lowered



Dicoumarol

prothrombin level could be restored to normal, and the hæmorrhagic tendency counteracted, by administration of alfalfa (containing vitamin K) in the diet. The active toxic factor in spoiled sweet clover was obtained in crystalline form by Campbell and Link (1941) and it was chemically identified by Stahmann *et al.* (1941) as 3 : 3′-methylene-*bis*-4-hydroxy-coumarin (Dicoumarol).

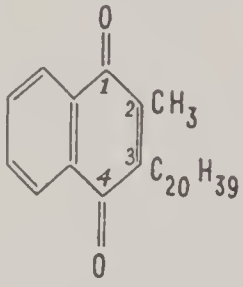
Dicoumarol is a white microcrystalline powder, which is only

slightly soluble in water. It is prepared synthetically and is cheap to produce.

Mode of Action of Dicoumarol

(1) Dicoumarol selectively depresses the formation of prothrombin by the liver, other functions of the liver remaining unaffected. This effect is entirely similar to that produced by vitamin K deficiency, and it may well be that dicoumarol is a competitive inhibitor of vitamin K whose formula may be seen to resemble that of half the dicoumarol molecule (see p. 335). The consequent reduction in plasma prothrombin level prolongs the prothrombin clotting time, and when this is sufficiently increased the ordinary clotting time of blood is also prolonged (Quick, 1944).

Moloney *et al.* (1948) have studied the clotting time of blood



Vitamin K

(2-methyl-3-phytyl-1 : 4 naphthaquinone)

collected in silicone coated ("drifilmed") tubes and have found that in patients who have received dicoumarol the clotting time might be greatly prolonged (e.g. from the normal time of 30–60 minutes to 24–72 hours), even though the clotting time, as measured in ordinary glass tubes, showed no significant change. They suggest that the silicone tube method gives much more valuable information about the prevention of intravascular clotting than the ordinary method, and the former may indeed be more accurate than the estimation of plasma prothrombin levels in the control of dicoumarol dosage. Unfortunately, the silicone tube technique is at present too complicated for routine use.

(2) Dicoumarol reduces the adhesiveness of platelets and so helps to prevent thrombus formation.

(3) It prolongs clot-retraction time.

(4) Dicoumarol has no other pharmacological actions and death from toxic doses is due to hæmorrhage.

It has been suggested by Link *et al.* (1943) that dicoumarol might be converted to salicylic acid in the body. They showed that sodium salicylate reduced the plasma prothrombin level in rats, but that in this respect it was much weaker than dicoumarol. Other workers have confirmed these results in man and suggest that the bleeding which may occasionally follow massive therapeutic dosage with salicylates, is due to hypoprothrombinæmia. However, although the chemical structure of dicoumarol suggests that it might be broken down to salicylic acid, there is no evidence that this explains its mode of action.

Factors Which Influence Dicoumarol Action

(1) *Fever.* Richards (1943) noted considerable enhancement of the action of dicoumarol on prothrombin time in rats in which fever had been artificially induced. This factor should be remembered in the therapeutic use of the drug.

(2) *Liver and Kidney Damage.* Richards and Steggerda (1943) found that carbon tetrachloride, in doses which had no significant effect on prothrombin levels, greatly sensitized rats to the action of dicoumarol, presumably by production of slight liver damage. They also showed that bilateral nephrectomy prolonged and increased the prothrombin lowering action of dicoumarol, and suggested that dicoumarol itself, or active breakdown products, might be excreted via the kidney. Thus it is clear that severe hepatic or renal disease might greatly enhance the toxicity of dicoumarol. For example, Reisner *et al.* (1949) found that in patients with liver disease or congestive heart failure, dicoumarol produced a greater fall in plasma prothrombin level than in normal persons.

Administration and Duration of Action of Dicoumarol

Dicoumarol is now always given by mouth, though Bingham *et al.* (1943) originally showed that the disodium salt was effective intravenously. By either route the onset of action is delayed for 12–72 hours and persists for a further 72 hours or more. Fig. 37 shows the duration of action of different single doses of dicoumarol (Lehmann, 1942).

Estimation of Prothrombin in Dicoumarol Therapy

The response to dicoumarol is very variable and it is necessary to control its dosage by daily determinations of the plasma prothrombin level. The one-stage technique described by Quick (1942) has been extensively used for this purpose. Briefly, the method consists of adding optimal amounts of brain thromboplastin and calcium to undiluted or 12.5 per cent oxalated plasma, so that, in the presence of the usual amounts of fibrinogen, the

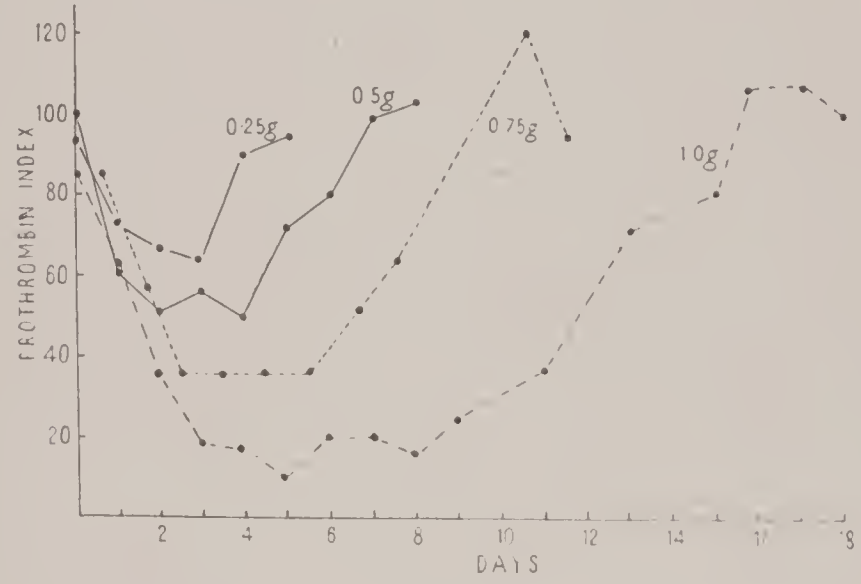


FIG. 37

HYPOPROTHROMBINÆMIA AFTER DICOUMAROL

Effects of different doses. Note the prolonged duration of action. Ordinate = Prothrombin Index (as percentage of normal = 100 per cent). Abscissa = time in days.

(After Lehmann, 1942.)

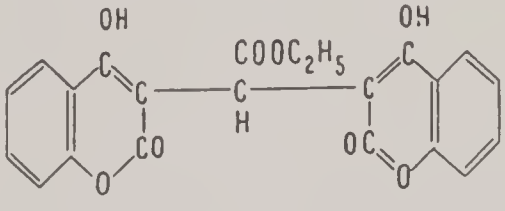
rate of clotting of the mixture is proportional to the concentration of prothrombin. The clotting time, measured in seconds, is translated into prothrombin concentration, expressed as a percentage of the normal, by reference to calibration curves derived from tests on samples of normal plasma in which the prothrombin concentration has been reduced by dilution. Unfortunately different samples of brain thromboplastin give different results, so it is difficult to compare one worker's results with another's. The aim with dicoumarol therapy is to reduce the prothrombin level to between

10 and 30 per cent of the normal. Below 10 per cent, bleeding is very liable to occur.

Biggs and Macfarlane (1949) have reviewed and studied the various factors which influence Quick's one-stage method of estimating prothrombin concentration. Though the method is theoretically inferior to the two-stage method, they regard it as of proved clinical value. Biggs and Macfarlane (1949) have compared the use of Russell viper venom+lecithin as thromboplastin, with the original brain thromboplastin used by Quick. They found that with Russell viper venom the test was much less sensitive, and in many instances more irregular, than with brain thromboplastin. Fig. 38 shows that in one patient, when Quick's technique showed a reduction of prothrombin concentration to 5 per cent of normal, and haematuria had occurred, a simultaneously performed test employing Russell viper venom+lecithin as thromboplastin, gave a prothrombin concentration of 35 per cent of normal. James (1949) has recorded similar findings.

The Fate of Dicoumarol in Man. Weiner *et al.* (1949) have studied the fate of dicoumarol in man. The rate of its breakdown depended on its level in the plasma; at high plasma levels a smaller proportion was destroyed than at low plasma levels, but there were large individual variations. About 99 per cent of dicoumarol in the plasma was bound to proteins. Only traces of dicoumarol were found in the urine.

Actions of a Dicoumarol Derivative ("Pelentan," "Tromexan")



3 : 3′-Carbethoxymethylene-*bis*-4-hydroxycoumarin ("Tromexan")

This compound was introduced in Czechoslovakia in 1945, and its use has more recently been described in Switzerland (Reinis and Kůbík, 1948; von Kaulla and Pulver, 1948; Della Santa, 1949).

The main features of its actions appear to be :—

(1) It has about $\frac{1}{4}$ the potency of dicoumarol, but is proportionately less toxic.

(2) Its hypoprothrombinæmic action develops, and passes off, more rapidly than that of dicoumarol. Its action commences

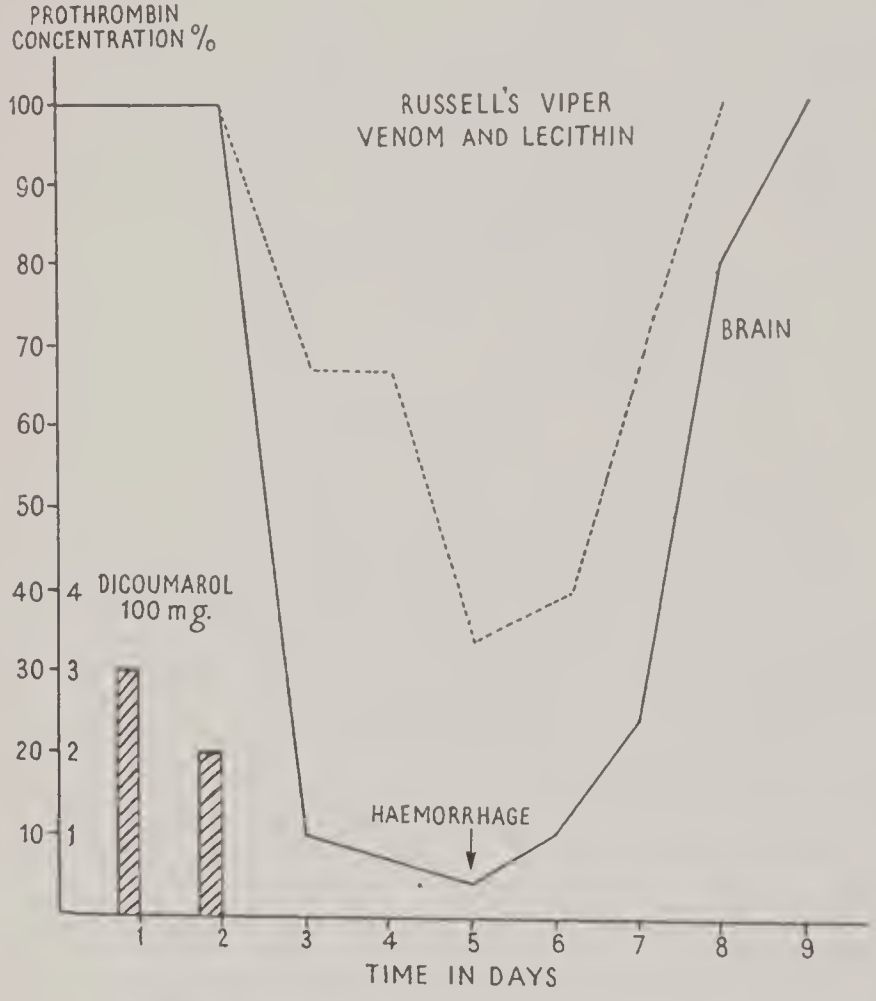


FIG. 38

COMPARISON OF THROMBOPLASTINS IN PROTHROMBIN ESTIMATION

The ordinate shows prothrombin concentration expressed as a percentage of normal. It can be seen that after dicoumarol administration, the Quick test, using brain as the source of thromboplastin, shows a much more marked hypoprothrombinæmia than when Russell's viper venom and lecithin are used as thromboplastin.

(After Biggs and MacFarlane, 1949.)

within 2–3 hours of oral administration and reaches its peak in 12–24 hours; after establishment of its full action, cessation of administration is followed by a 40 per cent increase in prothrombin level within 24 hours, and a return to normal levels within 48 hours.

(3) "Tromexan" is more rapidly metabolized and excreted than dicoumarol.

(4) The actions of "Tromexan" are enhanced and prolonged in the presence of liver damage.

(5) The daily dosage of "Tromexan" is at first 1,200 mg. (best given in four divided doses); later 300–600 mg. daily maintains the action without producing cumulative effects, though frequent measurements of prothrombin concentration are still necessary.

(6) Its clinical effectiveness in thrombotic conditions is comparable to that of dicoumarol.

(7) Hæmorrhagic complications have followed overdosage with "Tromexan."

Burt, Wright and Kůbík (1949) have recently reported on the clinical use of this compound in 126 patients with venous thrombosis, pulmonary emboli and arterial thrombosis or emboli. The drug was given for periods ranging from 5 days to 10 months. In over 80 per cent of the patients who received adequate dosage, the prothrombin level was reduced to less than 50 per cent of normal within 36 hours of administration of the first dose, and after withdrawal of the drug recovery was equally rapid. The toxic effects were slight nausea and vomiting in a few patients (perhaps due to the bitter taste of the tablets), and transient bleeding in 3 cases, in one of whom an over-dose was given by mistake.

It certainly looks as if "Tromexan" represents a distinct advance on dicoumarol, though its administration still needs to be controlled by frequent plasma prothrombin estimations.

General Uses of Anticoagulants

Heparin has been used for a variety of purposes. It is frequently used in experimental animal work when avoidance of clotting in arterial and venous cannulæ is particularly important; it has been widely used to prevent blood clotting *in vitro* where its very low osmotic pressure helps to avoid shrinkage of erythrocytes. In relation to blood transfusion it has been used both *in vitro* and

in vivo to prevent clotting of the donor's blood. Wiener and Wexler (1946) have used heparin to prevent the clotting of infant's blood during the course of exchange transfusions for erythroblastosis.

Anticoagulants in the Treatment of Thrombosis

Intravascular clotting, usually termed thrombosis, occurs in many circumstances in the course of diseases of medical and surgical interest. The danger to life from pulmonary embolism, and the chronic ill-health resulting from widespread venous obstruction in the lower limbs, illustrate different aspects of this important problem. The prevention or limitation of such thrombosis by means of anticoagulants has been much studied of recent years and some impressive results have been recorded.

Prevention of Thrombosis in Animal Experiments

Experimental animal work in this field began in 1932 in the Department of Surgery of the Toronto General Hospital as an extension of the chemical work on heparin done at the Connaught Laboratories of the University of Toronto. Murray *et al.* (1936, 1937) showed that in dogs heparin could prevent thrombosis which otherwise followed mechanical or chemical (sodium ricinoleate) injury to veins. Best *et al.* (1937) also demonstrated that heparin could prevent agglutination of platelets and white thrombus formation in a collodion or cellophane loop connecting an artery to a vein. Kolff in his book "The Artificial Kidney" (1946) has likewise described the successful use of heparin to prevent thrombus formation in the cellophane tubing used in his apparatus.

Solandt and Best (1938) found that the production of coronary thrombosis in dogs by intra-arterial injection of sodium ricinoleate, could be prevented by previous administration of heparin; these authors, with Solandt *et al.* (1939), also showed that heparin could prevent the development of artificially induced cardiac mural thrombi.

Clinical Use of Anticoagulants in Thrombosis

Decubitus Thrombosis. The commonest type of thrombosis for which anticoagulants have been used is that described by Le Quesne (1948) as "*Decubitus Thrombosis.*" This term describes

the thrombosis which originates in the legs following operations, after childbirth, or in the course of certain medical diseases, particularly in old people. Thrombosis in this state occurs first, largely as the result of stagnation, in muscle veins in the lower part of the leg, and subsequently spreads by deposition in the direction of the blood stream so that eventually it may form a long eel-like clot, with a smooth slippery surface, extending up to the femoral vein. At first this clot is anchored only at its lower end, it is friable, and rupture releases a portion which is carried away by the blood stream to produce pulmonary embolism. If the clot does not break it thickens to occupy the lumen of the vein, and inflammatory processes lead to its organization, with subsequent development of the well-known signs of venous obstruction (phlegmasia alba dolens) (Bauer, 1946).

Wright (1948) has studied the blood and circulatory changes which follow operations. She observed an increase in the number of platelets and an increase in their stickiness, an increase in plasma prothrombin concentration and a prolongation of the leg-carotid circulation time. These changes first became evident about 3 or 4 days after operation and reached their peak after 10–12 days, which is also the time when venous thrombosis (and pulmonary embolism) is most likely to occur. All these abnormalities may be corrected by anticoagulant therapy.

In theory, it would of course be desirable, and some have tried in practice, to use anticoagulants prophylactically to prevent the development of the initial clot, but there are some objections to such a course, and it has been found more feasible to administer these substances as early as possible after diagnosis of the original thrombosis. Anticoagulants, by preventing the extension of the clot, decrease the likelihood of embolism and restrict the consequences of venous obstruction. For example Bauer (1946) has compared the results at the Mariestad Hospital, Sweden, in a large series of cases after the introduction of heparin, with those of a large series previously studied. He records the following figures, which are presented in Table 12.

Zilliacus (1946) has reviewed the effects of anticoagulant therapy (mainly heparin) on the course of thrombosis and pulmonary embolism in a series of Swedish hospitals during the years 1940–45; his work supports and supplements the results recorded by Bauer.

TABLE 12

	No Heparin	Heparin treated
Mortality rate in cases with thrombosis	18%	1.4%
Average duration of stay in bed	40 days	4.7 days
Serious after effects, e.g. ulceration of legs	++	±

Post-operative Thrombosis. Barker *et al.* (1947) have described the results of combined heparin-dicoumarol administration in patients with post-operative and other types of thrombosis. Among patients with signs of pulmonary embolism or infarction the frequency of fatal embolism was reduced from 18 per cent to 0.3 per cent, and in patients with signs of thrombophlebitis the frequency of fatal pulmonary embolism was reduced from 5.7 per cent to zero.

Ball and Hughes (1949) have recorded the effects of heparin and dicoumarol in 100 patients with thrombosis and/or embolism. Fig. 39 illustrates the prothrombin response during anticoagulant therapy after a cardiac infarction in a patient with deep calf thrombosis.

Coronary Thrombosis. Anticoagulants have recently been used in the treatment of *coronary thrombosis with myocardial infarction.* They may help in four ways :—

(1) By preventing the spread of thrombosis in the coronary arteries.

(2) By preventing the development of mural thrombosis at the site of infarction.

(3) By preventing the development of decubitus thrombosis in the legs.

(4) By increasing blood flow through the coronary arteries (Gilbert and Nalefski, 1949).

The effects of dicoumarol in 432 cases were compared with 368 control cases by Wright *et al.* (1948) who recorded a statistically significant reduction of mortality rate from 24 per cent to 15 per cent during the period of anticoagulant therapy (see also Chap. 17).

Administration and Dosage

Heparin. When heparin is given alone in post-operative thrombosis, Bauer (1946) recommends a dose of 150 mg. intravenously 4 hourly for 2 or 3 doses, then 150 mg. morning and night + 100 mg. once or twice during the day, and later when the symptoms have subsided 100 mg. twice a day. Others have suggested that the dosage of heparin be related to the coagulation time, which should be prolonged to about three times normal (Reich and Eisenmenger, 1948). This is usually attained with 50–100 mg. every 4–6 hours.

Heparin in Pitkin's menstruum should be injected subcutaneously in doses of 200–400 mg. every 48 hours.

When heparin is combined with dicoumarol it (i.e. the heparin)

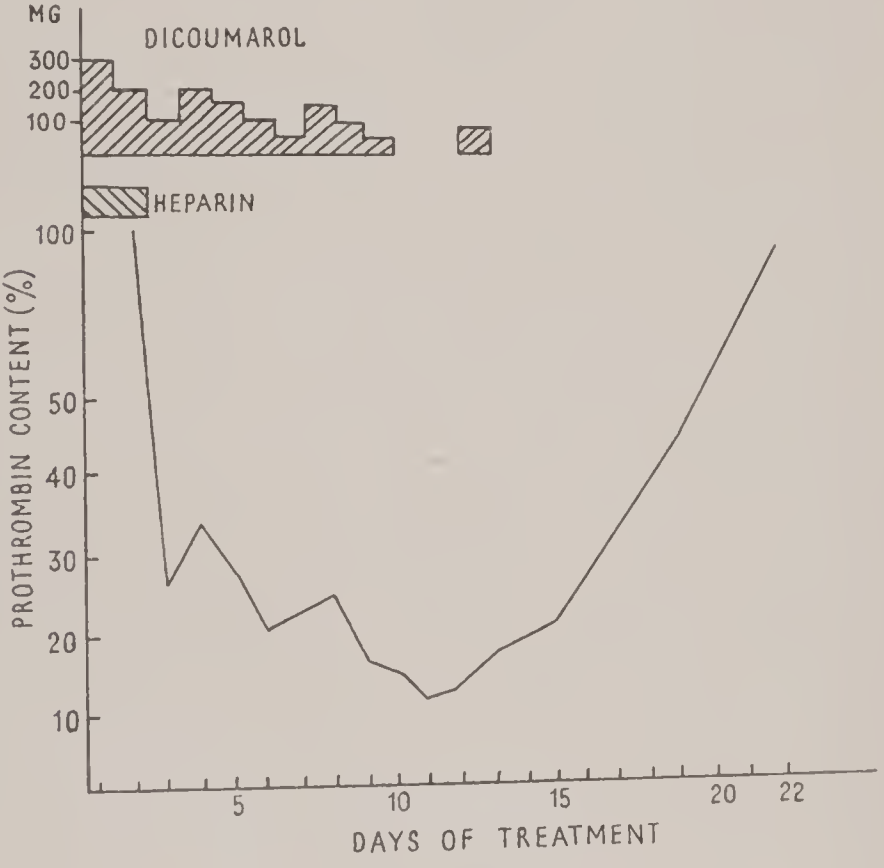


Fig. 39

This chart shows the effects of combined *Heparin-Dicoumarol* therapy in a patient with deep calf thrombosis following cardiac infarction.

(After Ball and Hughes, 1949.)

is only given during the first 36–48 hours of treatment, until the dicoumarol has had time to work, and most workers, e.g. Barker *et al.* (1947), recommend 50 mg. 4 hourly by intravenous injection for this purpose.

Dicoumarol. Dicoumarol is given in capsules by mouth. It is usual to give 300 mg. on the first day, 200 mg. on the second day and to regulate subsequent doses by daily measurements of the prothrombin level which should be reduced to about 20 per cent of the normal value in the plasma. If the prothrombin concentration exceeds 20 per cent of normal, 200 mg. of dicoumarol is given on that day, if it is between 10 and 20 per cent of normal the dicoumarol dose is 50–100 mg., and if the prothrombin level is less than 10 per cent of normal no dicoumarol is given on that day (Barker *et al.*, 1947 ; Wright *et al.*, 1948). The daily total is best given in one dose. The duration of treatment is related to progress, except in patients with coronary thrombosis to whom dicoumarol should be given for 30 days after the last thromboembolic episode. The dosage of tromexan is given on p. 312.

It cannot be stressed too emphatically that *dicoumarol should never be given without laboratory control of the plasma prothrombin level* ; fatal hæmorrhage has resulted in patients in whom this precaution has been ignored (Duff and Shull, 1949). When dicoumarol is first given to patients with thromboembolic complications, daily determinations of the prothrombin level are required before each dose of the drug. It is feasible to give dicoumarol over long periods of time, and Olwin (1949) has reported administration for up to 23 months ; he found that after initial stabilization it was quite safe to measure the prothrombin level at weekly intervals. Olwin noted marked variations in individual requirements to maintain a prothrombin level between 10 and 30 per cent of normal ; for example one patient required 100 mg. of dicoumarol weekly, whereas another was not satisfactorily controlled by 2,800 mg. weekly.

Antagonists to Anticoagulants

The effects of *heparin* may be immediately antagonized by intravenous injection of 5–10 ml. of 1 per cent protamine sulphate, for reasons previously explained (p. 304), or more slowly by blood transfusion.

The actions of *dicoumarol* may be antagonized :—

(1) By intravenous injection of 60–75 mg. of menadione bisulphite (water soluble vitamin K preparation), every four hours. This antagonizes the depressant action of dicoumarol on prothrombin formation in the liver, but probably takes at least 12 hours to become effective. James *et al.* (1949) have shown that the oxide of 2-methyl-3-phytyl-1: 4-naphthoquinone (vitamin K_1 oxide) works more rapidly than either menadione sodium bisulphite, or

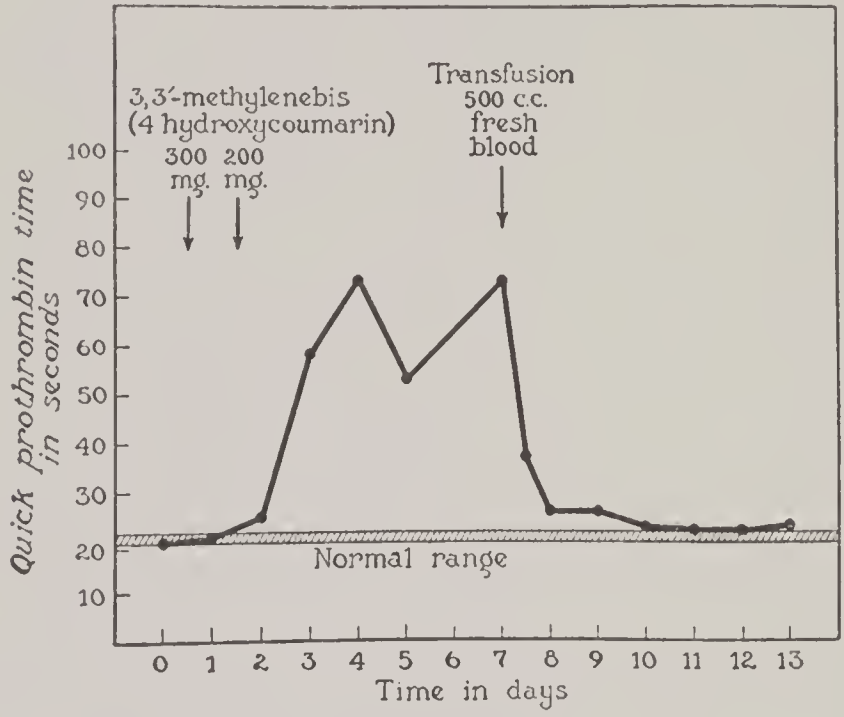


FIG. 40

Effect of transfusion of fresh blood on prothrombin time prolonged by dicoumarol.

(After Allen, Barker and Waugh, 1942.)

the tetrasodium salt of 2-methyl-1 : 4-naphthohydroquinone diphosphoric acid ester (synkavite), for this purpose.

(2) By transfusions of fresh whole blood, which provide small quantities of prothrombin without delay (Fig. 40).

Toxic Actions of Anticoagulants

Hæmorrhage is clearly the most probable complication of the use of anticoagulants. The danger is greater with dicoumarol than with heparin owing to the much more prolonged action of the

former. However, if the dosage is properly controlled serious bleeding with dicoumarol occurs in only 1–2.5 per cent of cases (Barker *et al.*, 1947), though minor hæmorrhages, e.g. epistaxis, local ecchymoses and transient hæmaturia may occur more frequently. Dicoumarol does not increase capillary fragility, but the bleeding time may be prolonged (Thorsén, 1947).

Other toxic effects with these substances include very occasional allergic hypersensitivity to heparin and to dicoumarol, and the latter sometimes produces headache, nausea and diarrhoea.

Contraindications to the Use of Anticoagulants

Blood diseases with purpura, fresh wounds and open ulcers are clear contraindications to the use of these drugs. It is now recognized that these substances are dangerous in subacute bacterial endocarditis, in which they have previously been used (particularly heparin) to enhance the actions of sulphonamides and penicillin.

Dicoumarol should not be given to patients with kidney or liver damage.

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CHAPTER 15

THE MODE OF ACTION OF DRUGS

There has been much investigation concerning the mechanism by which drugs produce their action in the body. Whatever their method of administration, they are distributed in a variety of ways, and ultimately reach the cells and the action is then due to the effect on cells. Drugs can produce their effects either by acting on the *surface* of the cell or by penetrating into the cell and producing some effect in the *interior* of the cell. The cell consists of a nucleus and surrounding cytoplasm and the effect of a drug can thus be exerted either on the cytoplasm or on the nucleus (or both). There is indeed much evidence that certain substances do penetrate into the interior of the cell, and in fact cells have the capacity to accumulate certain substances in their interior producing a concentration which is much higher than that present in the surrounding medium. One mechanism by which drugs can produce their effect is by preventing the concentration of certain substances in the interior of the cell. It seems likely that the action of penicillin is of this type, preventing the accumulation of glutamic acid in certain cells (see p. 148).

Theoretically an action on the *nucleus* might be exerted either by the drug penetrating into the nucleus and producing some effect, or by producing an effect on the surface of the nucleus, i.e. at the nuclear membrane. Such effects have not so far been studied, though a beginning has been made by investigating the structure of the nuclear membrane by means of the electron microscope (Callan *et al.*, 1949). There have been some investigations on the actions of drugs on the nucleus, e.g. in the case of substances like colchicine which inhibits mitosis, or other substances which have actions on the chromosomes and genes (see Chap. 11, and also discussion by Dustin, 1947). But on the whole the investigation of the action of substances on the nucleus is in a very early stage and requires much further work. It is also possible that substances produce effects at other

surfaces in the cell, e.g. at the surface of the nucleolus, Golgi apparatus, etc.

Recent developments in pharmacology have concentrated attention on the action of drugs at two sites, viz. (1) at the surface of cells, i.e. action on membranes, and (2) on enzymes. It is true that an action at the cell surface will frequently produce its ultimate effects by involving an enzyme system. Much has been learned about the properties of the cell surface and it seems likely that specific areas exist capable of combining with certain substances, and that such combination is followed by action on the cell.

Modern pharmacology has seen the development of the concept of Ehrlich that drugs can unite with *specific receptors* and that this occurs only when the drug and the receptor fit one another in the same kind of way as a key fits into a lock, though it must be emphasized that we know very little about the nature of these receptors and their physico-chemical constitution. It seems likely, however, that such receptors can at least be part of, and localized on, the surface of the cell, or they may be part of an enzyme system. A further development of this idea is that a receptor may be occupied not only by a specific substance which fits accurately on to it, but also by other substances rather similar in chemical constitution, which also fit on to the receptor, though less accurately; if the receptor is occupied by its appropriate *specific substance* which is present in the surrounding medium or substrate, then changes occur which ultimately affect the activity of the cell. For example, a receptor in a muscle cell or gland may become occupied by a substance such as acetylcholine, and this is then followed by an action on the cell, e.g. contraction of the muscle, or activation of glandular secretion. The receptor may, however, become occupied by some *closely similar chemical substance* present in the surrounding medium or substrate: no further action then occurs and the effect, e.g. on the muscle or gland, is not produced. But there is a further important consequence of this. If the receptor is occupied by a substance which is inactive, then the appropriate chemical substance can no longer become attached to it, even if it is present in the surrounding medium. Two substances present in the surrounding medium (or substrate) can therefore compete for a receptor, and the term *substrate competition* has been applied to this.

Properties of the Cell Membrane

There are many theories concerning the nature of the cell membrane, including the one that it does not exist. The penetration of various substances into cells is a very complex question. In addition, any theory about the cell membrane must explain certain other properties it possesses, notably its surface tension and wetting properties. In all probability the membrane contains lipoid, cholesterol and protein, possibly with the lipoid inside and the protein outside, as suggested by Danielli (1937). It seems likely that some of the protein in the cell membrane forms a part of enzymes, i.e. that it makes up the protein part of some enzymes. This would explain the very great speed with which action on cells can be produced, since the enzymes are part of the cell membrane and are thus readily accessible. Moreover drugs which are adsorbed on to the cell surface may greatly change the surface properties, e.g. pressure and potential, and this may markedly affect the rate at which chemical reactions can occur at the cell surface.

Substances can penetrate inside the cell (i) by simple diffusion through the membrane, either through an aqueous phase, or, by virtue of their lipoid solubility, through the lipoid layer of the membrane, (ii) by a process of active transfer involving the expenditure of energy (Höber, 1940). It seems likely that certain chemical groupings in organic substances have a particular affinity for the surface of the cell and do not readily remain in the aqueous phase. Höber has applied the term "organophilic-hydrophobic" to this part of the molecule. The first step in the physiological action and penetration of substances is probably their anchorage by such groups in the phase boundary between the cell and its aqueous surroundings. The substance may thus become attached to a movable carrier which then actively transfers it into the cell. No more precise description of this process of penetration is possible at the present stage of knowledge.

It seems very probable that certain drugs exert their actions on cells not by penetrating into them but by producing some effect on the surface. Drugs can be injected directly into the cells by the micromethod developed by Chambers, and in many cases the effect which is then produced is different from that which results when the cell is immersed in the drug. For example, narcotics and hydrogen cyanide do not produce a narcotic or lethal effect

when injected into Amœba Proteus, though such an effect results when the amœba is immersed in solutions of these drugs. It might be supposed that such drugs spread uniformly on the surface of the cell and affect all its parts equally. Clark (1937) has calculated the minimum number of molecules of certain drugs, e.g. adrenaline and acetylcholine, which will produce an effect on cells and has shown that the number is not sufficient to cover the surface of the cell. This also applies to the action of antibody on bacteria. These drugs presumably act by uniting with specific receptors which form only a small fraction of the total cell surface. In many cases, if not always, such specific receptors will form part of an enzyme system and the drug will thus, in the first place, produce its effect by modifying the activity of the enzyme.

It is noteworthy, as Clark has pointed out, that such specific receptors are found on surfaces much simpler than that of the cell. Metallic surfaces which have catalytic activity can be poisoned by small quantities of certain substances which act in very high dilutions on active patches of the metal surface responsible for the catalytic action.

The Nature of Enzymes

Enzymes play a vital part in controlling the activities of the body and the effects of drugs are largely brought about by their actions on enzymes. Some discussion of their nature and properties is therefore relevant at this point. Their main property is that they function as true catalysts and very markedly accelerate the rate of chemical reactions. Thus, for example, catalase decomposes more than two million molecules of hydrogen peroxide per minute at 0° C. per active centre of the enzyme. Enzymes may be regarded as one form of active or functional proteins. Such substances are composed of two main parts, viz. a large *carrier* part which is the protein, and one or more smaller *prosthetic* groups which are essential for the activity of the whole molecule. Hæmoglobin is a good example of such an active protein and is composed of these two groups, viz. the protein and the hæmin part of the molecule actually concerned in the taking up of oxygen. Antigenic substances may also be considered to be similar in nature and in this case the *hapten* moiety of the molecule is equivalent to the prosthetic group of the active protein. It seems likely too that the nucleoproteins which make up the chromosomes and genes can be

included in this class of substances and genetic methods may indeed prove of value in the investigation of the mechanisms by which they are produced in the cell.

In the case of enzymes we know that certain types of substances are essential for their activity by forming part of the enzymes. It seems likely that certain *metals* which are essential in animal nutrition owe their importance to the fact that they form part of an enzyme. Thus for example iron, so essential in the cellular metabolic processes, is a component of catalase, peroxidases, cytochromes and lactic dehydrogenases; manganese forms an integral part of arginase, etc. A number of *vitamins* too have been identified with the prosthetic group of an enzyme and it is possible, as Green has suggested, that all vitamins are so essential for the activity of the body because they form an intrinsic part of some enzyme system, and are needed for the continual renewal of the enzyme which has only a limited period of life. It is now known that nicotinamide, the anti-pellagra vitamin, is an essential component of cozymase which is the coenzyme of fermentation; riboflavine or vitamin B_2 is the prosthetic group of the yellow enzyme which was isolated from yeast by Warburg; the prosthetic group of carboxylase, which is concerned in the oxidation of pyruvic acid, is a diphosphoric ester of aneurin (vitamin B_1); and pyridoxal (vitamin B_6), in the form of its phosphoric ester, is the coenzyme of tyrosine decarboxylase. Moreover vitamin A, in the form of retinine, acts as a prosthetic group of the functional protein in the visual purple of the eye.

Many enzymes are known to be involved in the effects of drugs. In fact Green (1946) has gone as far as to postulate that "any substance which in trace amounts induces profound biological effects does so either by participating in, or by specifically affecting, some enzyme system." He gives a list of substances which produce effects in the body by an action on enzymes; to this may be added hesperidin and certain of its derivatives, which inhibit the action of hyaluronidase (Beiler and Martin, 1948) (see Table 13).

A similar mechanism is seen in the relation of anæsthetics to enzyme activity, as suggested by Quastel (1943). According to his theory anæsthetics reversibly inhibit a part of the respiratory enzyme system of the C.N.S., probably the flavoprotein component. It is likely, however, that this is only one factor in the action of anæsthetics.

In addition, of course, many enzymes are concerned in the *destruction* and *detoxication* of drugs. The list of enzymes known to be concerned in such processes is gradually increasing. An interesting recent addition is the finding that cozymase (diphosphopyridine nucleotide) is an important factor in the destruction of œstrone and testosterone by the liver (Coppedge *et al.*, 1948; Sweat and Samuels, 1948). Williams (1947), in his review of the metabolism of drugs in the body, shows quite clearly

Table 13

Pharmacological Agent	Enzyme inhibited by the agent
Fluoride	Enolase
One of the anterior pituitary hormones	Hexokinase
Cyanide	Cytochrome oxidase
Eserine	Choline esterase
Neostigmine	Choline esterase
Chlorinating agents	Triosephosphoric dehydrogenase
Iodoacetic acid	Triosephosphoric dehydrogenase
Ephedrine	Amine oxidase
Phlorhizin	Glucose phosphorylase
Gramicidin	One of the fermentation enzymes involved in phosphorylation
Hesperidin	Hyaluronidase
	Enzyme identical with agent
α-Toxin of Clostridium welchii	A lecithinase
Lytic factor of cobra venom	A lecithinase
Spreading factor	Hyaluronidase
One of the toxins of Cl. welchii	Collagenase

how often the enzymes concerned in these processes are still unknown.

Little is known about the mechanism by which these large protein molecules, enzymes, antigens and genes produce these highly specific effects. The configuration of their surface is highly important and this is no doubt partly dependent on the presence of prosthetic (or hapten) groups, the whole forming a pattern into which the substance acted upon fits, according to the lock and key analogy of Ehrlich. Recent work on the chemistry of proteins

suggests that their molecules are made up of chains of polypeptides which can be folded into a number of stable configurations. Pauling (1948) has developed the idea that the actual folding of the polypeptide chain is an important factor in determining the pattern of the active part of the protein molecule and that this can explain the specific action of enzymes, the formation of antibodies which interact specifically with the appropriate antigen, and the self duplicating power of genes and viruses. He points out that growing crystals are capable of selecting from a complex mixture of substances in solution just the molecules of one kind, rejecting all the others. Thus pure crystals of sugar may deposit from a jam in which there are molecules of many different substances. A similar type of mechanism may explain the formation of antibodies by antigens, and the formation of new molecules of genes and viruses. Enzymes, he believes, are molecules that are complementary in structure to the activated complexes of the reactions which they catalyse ; the attraction of the enzyme molecules for the activated complex would thus lead to a decrease in its energy, hence to a decrease in the energy of activation of the reaction, and finally to an increase in the rate of the reaction. These concepts are discussed in greater detail by Pauling in another article (1948) in which he suggests that the folding of polypeptide chains explains only the formation of antibodies and that other large biological molecules, such as enzymes, depend upon more deep seated changes in structure to produce these differences in specific properties.

Substrate Competition

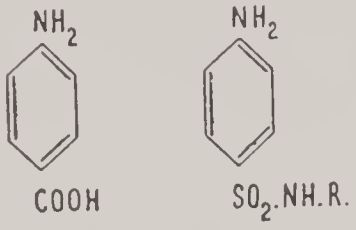
The general principles underlying the concepts of substrate competition have already been discussed (see p. 322). We must now consider how they can be used to explain the action of some drugs, and how they can be made a starting point for the discovery of new drugs.

It has been accepted for a long time that physostigmine and some related substances produce their main effects by a mechanism dependent on substrate competition, i.e. by competing with acetylcholine for the enzyme, cholinesterase (see Chap. 1). In the same way the action of ephedrine and perhaps other sympathomimetic amines is related to amine oxidase, one of the enzymes which destroys adrenaline in the body.

Sulphonamides and p-aminobenzoic Acid

New impetus was given to the development of this idea by the work of Woods and Fildes on the mode of action of sulphonamides (discussed in detail by Northey, 1948).

It was found that p-aminobenzoic acid, which is a natural constituent of some living materials, e.g. yeast, will inhibit the bacteriostatic action of sulphonamides on bacteria. As p-aminobenzoic acid and sulphonamides are similar in chemical constitution, Woods then postulated that sulphonamides compete with p-aminobenzoic acid for some enzyme receptors in the bacteria. In the presence of a sufficient concentration of sulphonamide the bacterial cell cannot take up p-aminobenzoic acid for its metabolic requirements. It is therefore deprived of this "*essential metabolite*" and becomes incapable of growth and reproduction; it thereupon falls a prey to the natural defences of the body.



p-aminobenzoic Acid Sulphonamide

There has been some discussion as to what exactly is meant by "essential metabolite." In connection with p-aminobenzoic acid the idea that was originally developed suggested that this substance is used in synthetic processes occurring during the anabolic activity of the bacterial cell; in other words, that p-aminobenzoic acid is eventually built up into the protein framework of the cell. Later evidence has indeed shown that p-aminobenzoic acid may be used in the synthesis of folic acid (Lampen and Jones, 1946) and that this synthesis can be inhibited by sulphonamides; moreover this inhibition is overcome in a strictly competitive manner by p-aminobenzoic acid (Nimmo-Smith *et al.*, 1948). The amounts of p-aminobenzoic acid which are present in living material and which are capable of inhibiting the action of sulphonamides are, however, minute, and barely sufficient to play a significant part in building up the framework of the cell. Green has suggested

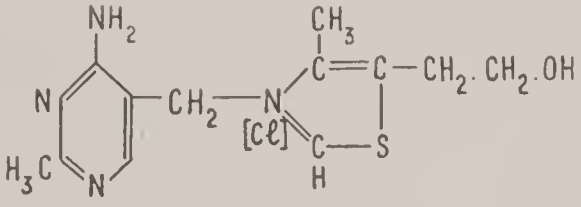
that essential metabolites, such as p-aminobenzoic acid, are used specifically in the building up of certain enzymes, and do not, like, for example, amino acids and fatty acids, take part in the general metabolic activities of the cell. According to this view, p-aminobenzoic acid forms part of the prosthetic group of an enzyme and is an essential metabolite by virtue of this function. This would account for the fact that only minute amounts are needed by the cell, and are capable of inhibiting the action of sulphonamides. If this hypothesis is accepted, p-aminobenzoic acid may still become part of the folic acid molecule, and the known actions of this substance are not incompatible with the possibility that it forms part of an enzyme system.

There has been a good deal of discussion as to why some sulphonamides are more active than others. Thus by some tests, sulphathiazole and sulphadiazine are about 200 times as active as sulphanilamide in producing a bacteriostatic action on some organisms. If the substrate competition theory is accepted, then it is reasonable to argue that the more closely the sulphonamide resembles p-aminobenzoic acid, the more capable will it be of competing with this substance for a specific receptor, and therefore the more active will it be. A number of investigations have suggested that the active form of the sulphonamide is the ionized form of the drug and that the more active substances like sulphathiazole owe their greater activity to their greater degree of ionization (Bell and Roblin, 1942). Various additions and modifications have been made to this theory (see Welch, 1945), but the question has not been fully clarified. Moreover, there is good evidence, discussed by Henry (1943) and by Sevag *et al.* (1943), that sulphonamides inhibit other enzyme systems, and it is quite possible that this may explain some discrepancies between the theories discussed above and the experimental observations. It is generally assumed that sulphonamides have no *specific* action on any bacteria and that the more active sulphonamides affect a large number of bacteria, including those which are comparatively resistant to these drugs, while the less active sulphonamides have a bacteriostatic action only on organisms which are comparatively susceptible to their action. An examination of the data, and especially of the relative actions of various sulphonamides on different organisms during the development of resistance, by no means completely supports this view and it is possible that here too alternative mechanisms of action may

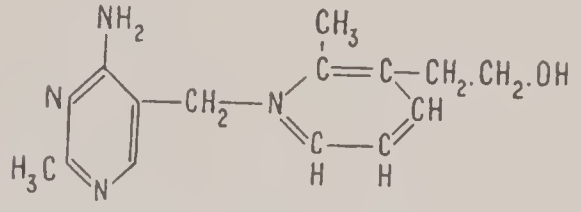
be concerned. The remarkable effect exerted by sulphones, e.g. sulphetrone, in leprosy is also difficult to explain on the assumption that substances of this group have no specific actions.

Other Antagonists of Essential Metabolites

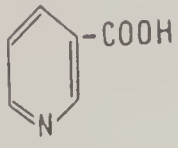
The concept relating the action of sulphonamides to p-amino-benzoic acid has proved very valuable and has been the starting point for the discovery of new chemotherapeutic substances. Search has been made for other substances essential for bacterial metabolism, and then for suitable analogues, which would compete with the essential metabolite in the metabolism of the bacteria and thus inhibit their growth and reproduction. It is known, for example, that aneurin, nicotinic acid and nicotinamide, and pantothenic acid are essential for the growth of certain bacteria, and the analogues, pyrithiamine, pyridine sulphonic acid and pyridine sulphonamide and pantoyltaurine were made and found capable of inhibiting the growth of the appropriate organisms. The relations between these various substances are shown in the formulæ below.



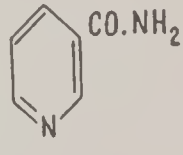
Thiamine (Aneurin)



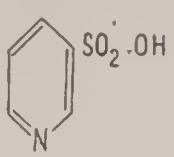
Pyrithiamine



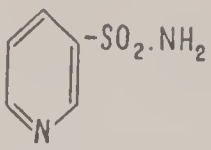
Nicotinic Acid



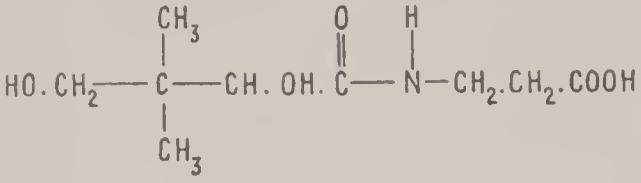
Nicotinamide



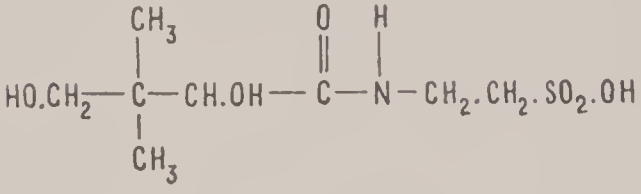
Pyridine Sulphonic Acid



Pyridine Sulphonamide



Pantothenic Acid

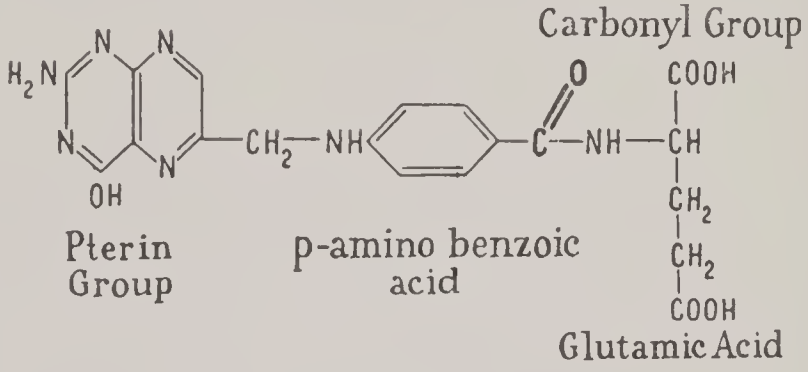


Pantoyltaurine

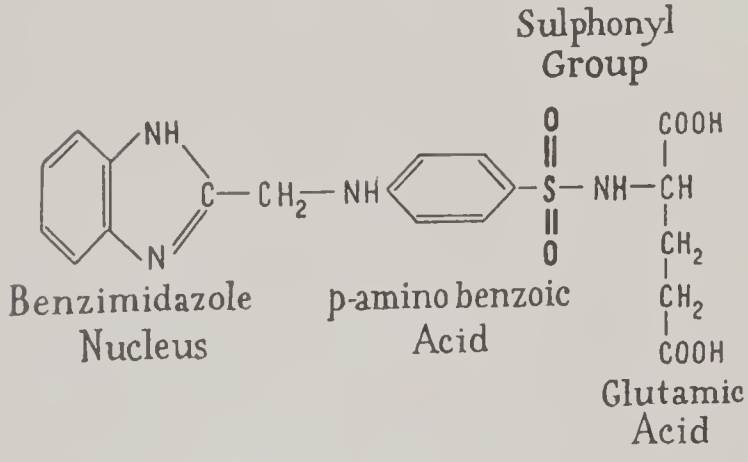
These results have not so far proved of any practical importance, although chemotherapeutic effects have been demonstrated in infected animals, e.g. by pantoyltaurine in rats, in which the blood level of pantothenic acid is sufficiently low to allow of inhibition by pantoyltaurine. This emphasizes the fact that an analogue which acts by substrate competition with a bacterial essential metabolite can be of value only when it does not appreciably affect the host, i.e. the effect on the invading organisms must be selective. This is well illustrated by the experiments with pyrithiamine, the analogue of vitamin B_1. This substance inhibits the growth of certain organisms which are dependent on an external supply of thiamine. But under suitable conditions it will also rapidly bring about symptoms of vitamin B_1 deficiency in the host, by competing with the uptake and utilization of aneurin. Thus the administration of pyrithiamine to weanling rats leads to the development of progressive inactivity, tremors, weakness and convulsions, and death ensues within some three days of the first appearance of the vitamin deficiency (Woolley and White, 1943). These effects are of course the same as develop in animals which are kept on a vitamin B_1 deficient diet.

Folic Acid Antagonists

Interesting antagonistic phenomena have also been obtained with derivatives of folic acid. This substance is essential for the growth of certain bacteria, e.g. streptococcus faecalis, and there is evidence that it plays a part in the synthesis of desoxyribonucleic acid (Prusoff *et al.*, 1948). Related substances produce an antibacterial action which is antagonized competitively by folic acid. For example, if the pteroyl nucleus is replaced by the benzimidazole nucleus, the resulting compound has still some growth promoting properties but if, in addition, the carboxyl group is substituted by a sulphonyl group, then the resulting substance (Derivative I, see below) becomes a metabolite antagonist and inhibits the growth of streptococcus faecalis (Edwards *et al.*, 1948). Inhibition of the growth of various organisms has also been obtained with simpler compounds, e.g. derivatives of the pteroyl part of the folic acid (Daniel *et al.*, 1948).



Folic Acid



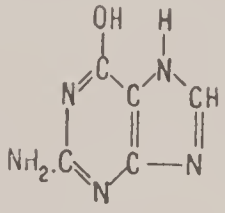
Derivative I

Another antagonist, which has been designated "7-methyl folic acid," though it has not been fully identified chemically, has also interesting properties. It antagonizes the growth of streptococcus fæcalis and produces various actions in animals. Rats receiving this substance develop symptoms typical of folic acid deficiency, i.e. loss of weight, reduction of hæmoglobin and white cell count, and necrotic and ulcerative changes in the mouth. Administration of folic acid reverses these effects (Franklin *et al.*, 1948). Another observation, which may open up an important field of investigation, has been made by Hertz (1948). He found that the growth of the chick's oviduct in response to œstrogen administration requires an adequate intake of folic acid. If chicks, maintained on a normal diet, are given "methyl folic acid" and treated with œstrogen, the growth of the oviduct is markedly inhibited. Additional administration of folic acid completely reverses this inhibiting effect. Similar effects have also been described in frogs (Goldsmith *et al.*, 1948), in mice (Hertz, quoted by Goldsmith *et al.*, 1948), and in rats (Hertz and Tullner, 1949). Moreover it has been shown that the response to testosterone of the seminal vesicles and coagulating glands in mice is interfered with by the administration of a folic acid antagonist (Goldsmith *et al.*, 1949). These results suggest that the enzyme systems responsible for the growth of certain sex-organs, which are stimulated by œstrogen or androgen, contain folic acid as a prosthetic group. Hertz and Tullner (1949) have also found that the response to œstrogen of the hen's oviduct is inhibited by an adenine analogue, 2, 6-diaminopurine: this effect is reversed by adenine (6-aminopurine) but not by folic acid. As these authors state, such observations should provide a useful experimental tool for the study of the basic metabolic mechanisms involved in growth processes in hormone-sensitive tissues.

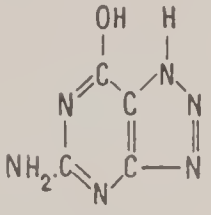
Significance in Malignant Disease. A similar line of attack might well lead to the control of growth of other tissues, and indeed Goldsmith *et al.* (1948) have found that the development of drosophila larvæ into adults is prevented by folic acid analogues. It is also possible that the administration of suitable antagonists might interfere with the growth of malignant tissue. Welch (1945) discusses a number of cases in which amino acids and related substances are competitively concerned in the growth of bacteria and animals. Sulphonic acid derivatives of various amino acids have been administered to mice with tumours in the hope of

inhibiting their growth, which is so much more rapid than that of the rest of the body, and which would therefore be expected to be more easily affected by an interference with the assimilation of amino acids ; unfortunately no appreciable effect on the growth of these tumours has been produced (Greenberg and Schulman, 1947).

It has, however, been shown that folic acid is essential for the growth of the Rous sarcoma in chickens, and that this growth can be inhibited by the administration of folic acid antagonists in doses which do not produce any symptoms of vitamin deficiency (Little *et al.*, 1948). The growth of a transplantable mouse sarcoma has



Guanine



Guanozolo

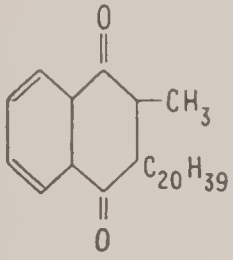
also been inhibited by the administration of a folic acid antagonist, 4-amino-N^{10}-methyl pteroylglutamic acid, also called A-methopterin (Moore *et al.*, 1949).

A number of purine analogues have also been made in the hope that they would be incorporated in the nucleoprotein and thus, perhaps selectively, inhibit the multiplication of certain types of cells. This has led to some very interesting results. It has been found that one of these purine analogues, called *guanozolo* (which is 5-amino-7-hydroxy-1H-v-triazolo [d] pyrimidine) produces very marked inhibition of growth of malignant disease in mice : so far this effect has been shown in three types of tumours, viz. a transplanted adenocarcinoma, a spontaneous mammary cancer and

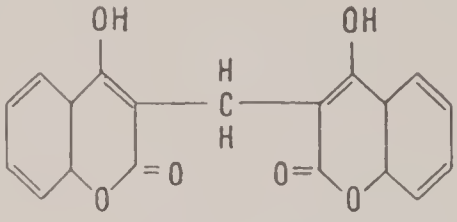
a lymphoid leukæmia. The treatment does not cause the death of the malignant cells but only checks their growth, as has been shown by pathological examination (Kidder *et al.*, 1949). The results of further experiments now in progress will be eagerly awaited, and their possible clinical significance is obvious.

Vitamin K and Dicoumarol

There are many other pharmacological actions which can be explained by the theory of substrate competition. In some cases the evidence is very good while in others it is at present only suggestive. It seems very likely that the action of dicoumarol (discussed in Chap. 14) is due to an interference with the function of vitamin K. This vitamin is essential for the formation of prothrombin (Warner *et al.*, 1936). It may thus, like some other vitamins already discussed, act as a prosthetic group in the enzyme



Vitamin K



Dicoumarol

system responsible for the synthesis of prothrombin. As can be seen from the formulæ shown above, the half molecule of dicoumarol is similar in chemical structure to vitamin K and probably acts by competing with it in the enzyme system. It is known that the effects produced by dicoumarol can be antagonized by the administration of large doses of vitamin K or of menaphthone, the synthetic analogue of vitamin K which has the same physiological actions (see p. 318).

Anti-histamine Drugs

It is possible that the action of anti-histamine drugs can also be explained by substrate competition, and this question is reviewed by Gaddum (1948). The clinical use of these substances is discussed in Chap. 3. In some cases, e.g. mepyramine, which is one of the most active of the anti-histamines, the resemblance to histamine

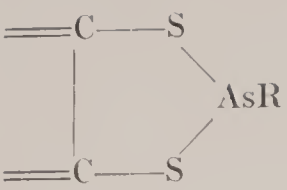
in part of the formula is quite striking, but with other substances, e.g. benadryl, the similarity is by no means convincing. The resemblance to histamine is still more strained, if existent at all, in the group of substances based on the pyridindene nucleus, e.g. thephorin (Lehmann, 1948).

If it could be shown that these anti-histamine substances protect histamine against destruction by the enzyme histaminase (diamine oxidase) this would support the view that they can compete for and occupy the same receptor. Kapeller-Adler (1949) has investigated this question and found that some anti-histamine substances, e.g. antistin, do actually exert such a protective action, whereas others, including the very active mepyramine, do not. All these findings suggest that the mode of action of the anti-histamines is more complex than merely simple competition for a receptor.

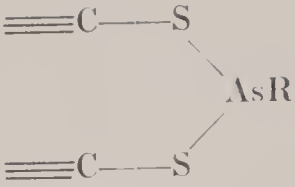
Dimercaprol (BAL)

One of the more interesting examples of substrate competition which has proved of practical value is that which involves BAL, or British Anti-Lewisite, so named by the Americans during the war. Unlike the other reactions so far discussed, BAL does not compete with the normal substrate for an enzyme receptor, but actually competes with the enzyme for the substrate. These relationships are illustrated in Fig. 41.

This work was made possible by the fundamental observations of Peters and his co-workers, and of Voegtlin and his colleagues, which suggested that *arsenicals* produce their toxic effects by reacting with certain essential thiol compounds present in protoplasm, probably —SH components of the pyruvate oxidase enzyme system (see Peters, 1948). These effects were demonstrated both *in vitro* and *in vivo*. This led to the search for substances with —SH groups which would compete with the —SH groups of the enzyme for the arsenical, but none of the thiol compounds tested at first, e.g. glutathione, diethyldithiocarbamate, proved to be of any value. Further work, however, suggested that lewisite and trivalent arsenicals combine with *two* essential —SH groups in the enzyme system to form stable arsenical rings of the type shown below, such compounds being more stable than compounds of an arsenical with two molecules of a monothiol compound.



Stable arsenical ring



Less stable compound of arsenical with two monothiol compounds

Stocken and Thompson (1946) prepared a number of dithiol compounds and tested their efficacy in antagonizing the action of lewisite. They found that 2 : 3 dimercaptopropanol, which is an oil with good power of penetration into tissues, was effective in

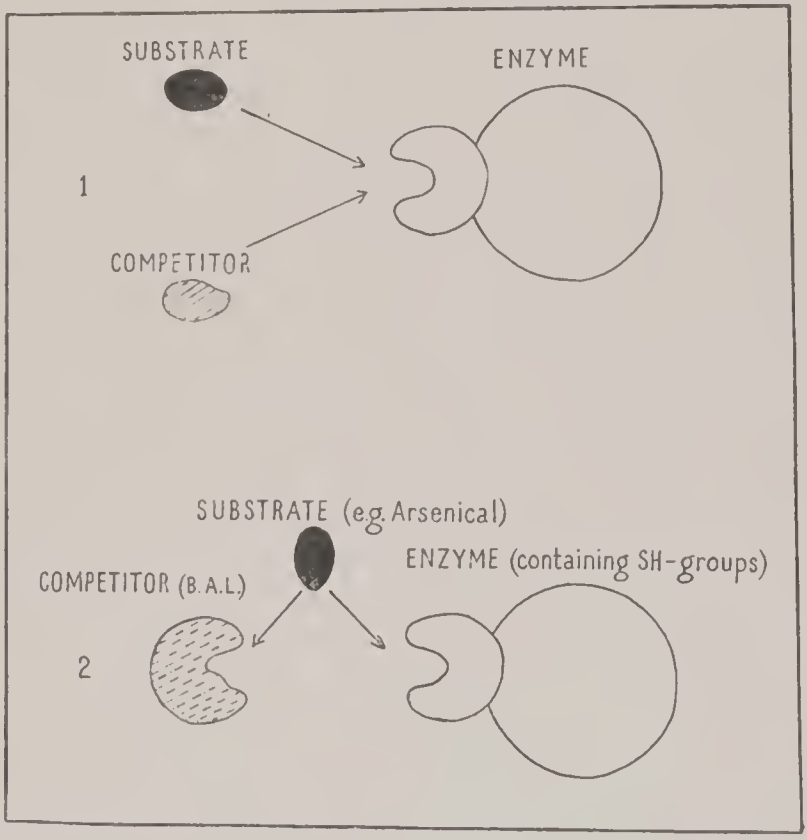
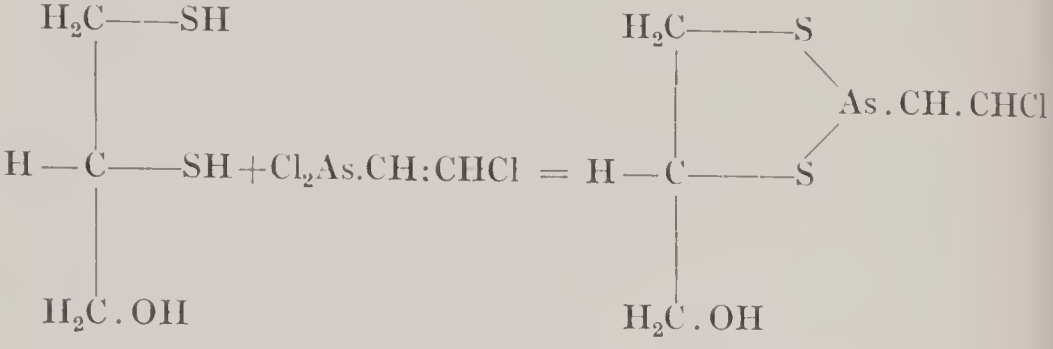


FIG. 41

DIAGRAMMATIC REPRESENTATION OF SUBSTRATE COMPETITION

(1) Two substances (e.g. p-aminobenzoic acid and a sulphonamide) competing for an enzyme receptor. (2) An enzyme receptor (containing two SH groups) and BAL (a dithiol) competing for a metallic poison (e.g. an arsenical).

preventing the vesicant action of lewisite on the skin. The reaction, which involves the union of two —SH groups with the arsenic, is as follows :—



BAL (2:3 dimercaptopropanol) Lewisite Stable ring compound

Further work showed that BAL can not only compete with the tissue thiol groups for the arsenical but can even reverse the reaction, provided it had occurred fairly recently, e.g. within about 1–2 hours. The treatment with BAL is followed by a marked increase in the excretion of arsenic in the urine (Stocken and Thompson, 1946). It is of interest that tissue cultures poisoned with lewisite largely recover when treated with BAL, showing that the effect can be exerted directly on cells (Fell and Allsopp, 1941). BAL not only forms stable compounds with arsenoxides, but also with a number of metals, e.g. gold, lead, bismuth and mercury, and might therefore be expected to be of value in the treatment of poisoning with these metals. Experimental evidence in rabbits shows that BAL is an effective antidote in acute poisoning caused by *antimony*, *bismuth*, *chromium*, *nickel* and *mercury*, but is ineffective against lead, thallium and selenium: indeed, in the case of lead and selenium BAL appears to produce an additive toxic action (Braun *et al.*, 1946). Weatherall (1948) finds that BAL glycoside does apparently reduce the mortality in animals poisoned with *lead acetate*, but concludes that the available evidence does not warrant the use of BAL in clinical plumbism. Similar results have been obtained by Germuth and Eagle (1948). They also found, however, that BAL strikingly increases the excretion of lead and believe that this justifies its cautious therapeutic trial in cases of lead poisoning. BAL is of value in protecting animals from the systemic effects of *cadmium*, but

appears to form toxic complexes with this metal which give rise to serious renal damage (Gilman *et al.*, 1946). Protection in rats poisoned with *gold* salts has been demonstrated by Gillmor and Freyberg (1948).

Experimentally it has been shown that BAL can give protection against substances other than metals, e.g. methyl-iodide (Buckell, 1949) and methyl naphthoquinone (Richert and Bass, 1949).

BAL is by no means entirely non-toxic and cannot be injected intravenously. A less toxic compound, a glycoside, has been prepared and named BAL-intrav., as it is suitable for intravenous injection (Danielli *et al.*, 1947). It is possible that this compound may prove of value in the treatment of the acute stages of metallic poisoning.

Clinical Use

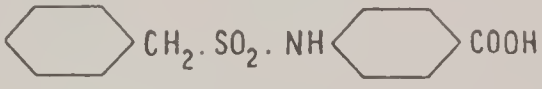
BAL is a colourless oil, soluble in fat solvents, and soluble in water to the extent of about 6 per cent. It is usually administered intramuscularly as a 5 per cent or 10 per cent solution in arachis oil and benzyl benzoate. The question of dosage requires consideration as BAL itself will produce toxic effects in amounts not far above the therapeutic level. In man, doses between 3 and 5 mg./kg. have produced some toxic reactions, but with single doses up to 8 mg./kg. the effects, which include lacrimation, salivation, vomiting and rise in blood pressure, last only a few hours. When applied to the skin BAL may produce irritation and wheal formation, and allergic sensitization to BAL does occur.

BAL is rapidly metabolized and excreted in the urine as a closely related thiol (Spray *et al.*, 1947). Other pharmacological actions are described in a supplement to the *Journal of Pharmacology*, 1946, 87, No. 4, and by Graham and Hood (1948). A method for the determination of BAL in blood is described by Aldridge (1948). According to Modell *et al.* (1946) no significant cumulation occurs in man when the drug is given in doses of 5 mg./kg. at three hourly intervals. In practice, therefore, BAL has usually been given, first at four hourly intervals, and after the first day, in twice daily injections. In Britain the doses have been of the order of 1.5 mg./kg., but higher amounts have been administered in the U.S.A. (Peters, 1947; Sulzberger and Baer, 1947). It can also be applied by inunction, in the form of an ointment, but is painful when applied to the inflamed skin (Longcope *et al.*, 1946).

BAL has been found of value in poisoning with *arsenicals* of various types, e.g. mapharsen, phenyl arsenoxide, lewisite. Striking effects, illustrated in Fig. 42, have been observed in cases of exfoliative dermatitis (Carleton *et al.*, 1948). BAL has been of value in poisoning with *mercuric chloride* and great importance is here attached to prompt treatment (Longcope and Luetscher, 1946). The drug has also been used to treat toxic reactions due to the administration of *gold* (*Lancet*, 1947), *lead* (Telfer, 1947), *bismuth* (Cormia and Blauner, 1947), *cadmium* and *zinc* (mentioned by Pfeiffer and Loew, 1947) and *tellurium* (Amdur, 1947; Mazzei and Schaposnik, 1949).

Carinamide (Caronamide)

This drug is used to decrease the excretion of penicillin and its therapeutic uses are discussed in Chap. 7. Its mode of action is interesting and apparently involves a competitive mechanism in the renal tubules. Of the penicillin excreted by the kidney, about



Carinamide

80 per cent is eliminated through the renal tubule and only about 20 per cent by the glomeruli (Beyer, 1947). This led to the search for drugs which would interfere with tubular activity and thus decrease the excretion of penicillin. Two drugs, viz. diodone (diiodopyridone acetic acid diethanolamine) (Rammelkamp and Bradley, 1943) and p-aminohippuric acid (Beyer *et al.*, 1944), were found to have such an effect, acting by competing for the transport mechanism. Carinamide (4′-carboxylphenyl-methane sulphonanilide) is even more specific in its action and apparently produces its effect by blocking the specific enzyme transport system responsible for the passage of penicillin through the tubular cells. Carinamide itself is not eliminated by the tubules and hence it is presumably taken up by the enzyme system, in competition with penicillin, but is not dealt with by the enzymes (Beyer, 1947). The tubular excretion of all four types of penicillin investigated (I, II, III and IV) is reversibly inhibited by carinamide (Beyer *et al.*, 1947).

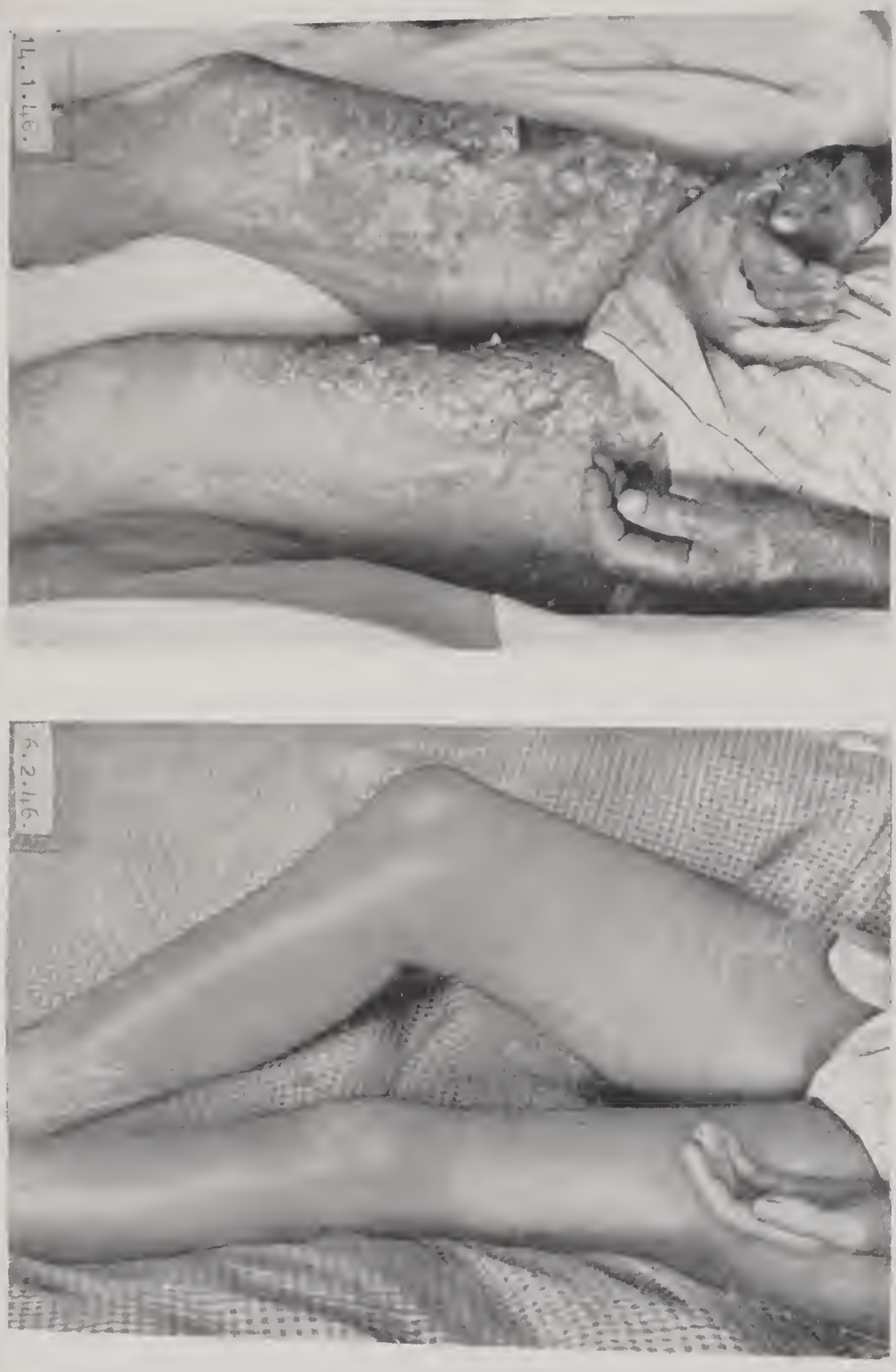


FIG. 42

Effect of treatment with BAL in exfoliative dermatitis following treatment with neoarsphenamine in a patient with secondary syphilis. 14.1.46. Condition immediately before the beginning of BAL administration (Full course given between 14.1.46 and 19.1.46). 6.2.46. Condition 23 days later.

(After Carleton *et al.*, 1948.)

[*To face page* 340.

The discovery of this drug will no doubt lead to the search for substances capable of inhibiting the secretory activity of other types of cells, by interfering with the appropriate enzyme system.

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CHAPTER 16

METHODS OF STUDYING THE FATE OF DRUGS IN THE BODY

One of the important advances in modern pharmacology is the progress that has been made in studying the fate of various substances in the body; this has involved the use of many new techniques. The findings have been of value not only in increasing our understanding of the mechanism by which drugs act, but also in the more successful application of drugs in therapeutics. A simple example will illustrate this point. Various methods have been devised for detecting and estimating sulphonamides in tissues and fluids. Such methods have been of value in studying the interaction between sulphonamides and p-aminobenzoic acid *in vivo*, and in showing that the antagonism between these two substances demonstrated *in vitro* also holds in the intact animal. They have therefore helped in elucidating the mechanism of action of the drug. In practice the estimation of sulphonamides in the blood, etc., has been of value in deciding whether, with the dosage given, the necessary chemotherapeutic concentration has been reached; it has also been shown that by the local application of the drug to certain tissues much higher concentrations can be produced locally, and thus lead to a very effective chemotherapeutic action. This has been well demonstrated for example in the treatment of ocular infections.

The measurements that have been made have thus given information about :—

(1) *The mode of action* of the drug, e.g. whether it is actually the active principle or whether it is changed in the body to some substance which is responsible for the effect produced. The various changes which occur in the body in pentavalent and trivalent organic arsenicals are good illustrations.

(2) *The site of action* of the drug. Sometimes this is a simple matter to determine, e.g. when a drug acts in the blood. But in other cases the matter is more difficult and considerable ingenuity

has been used in tracing drugs in various tissues. For example, acetylcholine containing radioactive nitrogen has been used to determine whether this substance penetrates through the membrane of nerves. It is remarkable, however, that even in well known fields of therapy information is sometimes lacking. There is, for example, little knowledge about the penetration of arsenicals into the eye, even though these substances are widely used in the treatment of syphilitic ocular lesions.

(3) *The concentrations* of drugs that are necessary to produce certain effects, and the period of time during which these concentrations must be maintained. Some drugs act in very low concentrations and hence the methods needed for their identification and assay must be very delicate. Very frequently, with the introduction of a new substance, it is necessary to devise methods for its assay in body fluids and tissues. Penicillin, streptomycin and proguanil are good examples of this.

The main methods which are used for the identification, assay and localization of drugs can be discussed under three headings, viz., physical, chemical and biological. It may be added that, though we have much knowledge about the distribution of drugs in the extracellular fluids, we know comparatively little about their penetration and distribution in cells and this must represent an important field of investigation in pharmacology.

(1) Physical Methods

The most important of these depends on the use of isotopes and this will be discussed in detail below. Other methods too are highly sensitive and have given interesting results.

Some substances show *fluorescence in ultra-violet light*, e.g. mepacrine and quinine, and this has been used for their localization in tissues. Helander (1945) has used this method for detecting the presence of various sulphonamides in different organs. Beautiful microphotographs show the presence of sulphonamides in kidney cells as well as in the lumen of the tubules, and also illustrate the affinity of one of these drugs, prontosil, for collagen and elastic fibres; high concentrations of prontosil are thus produced and stored in the lungs, tendons, skin, ligaments and elastic cartilage.

Brachet (1947) discusses some recently developed methods for the estimation of small quantities of substances. Caspersson (see Brachet, 1947) has elaborated a physical method for detecting

nucleic acids in the cell. These substances have an absorption band in the ultra-violet light with a maximum at about 2,650 Å. Caspersson has measured the absorption spectrum, in ultra-violet light, of particles of a diameter as small as 0.1 μ and has thus been able to determine the distribution of nucleic acids in the cell nucleus and cytoplasm under various conditions. The results have been of particular interest in the study of the rôle of nucleic acid in cell division. A similar method could be used for other substances which have a definite absorption spectrum.

The *polarographic* method, which was developed by Heyrovsky more than 20 years ago, is now being applied to the assay of substances of biological interest e.g. steroids. The method is based on the interpretation of the current voltage waves that are obtained when solutions of electro-reducible or electro-oxidisable substances are hydrolysed in a cell in which one electrode consists of mercury falling in drops from a very fine bore capillary glass tube. From the characteristics of such curves both the type of substances present in the solution and their concentration can be determined. The method is specially suitable for small concentrations and since the analysis can be performed with a very small volume of solution, mere traces of substances can be determined. The subject is discussed in detail in the book by Kolthoff and Lingane (1946).

(2) Chemical Methods

These are in general a good deal less sensitive than the physical or biological methods. Very often the substances are estimated after suitable extraction from tissues or fluids. Chemical reactions leading to the development of a colour are frequently employed: the depth of the colour is most accurately determined by means of photo-electric methods.

Methods for estimating very small quantities of substances have been elaborated by Linderstrom-Lang and Holter, and by other investigators, and are discussed in the book by Brachet mentioned above. With micro-titration methods, for example, amounts of ammonia of less than 0.3 μg. can be estimated with an accuracy greater than ± 0.005 μg. Colorimetric and volumetric methods capable of being applied to very small quantities of material have also been developed. For example, Heatley *et al.* (1939) have described a micromanometer in which 1 mm. on the scale corresponds to 0.004 to 0.008 cubic millimeters of a gas.

Another method by which small quantities of substances can be demonstrated and roughly assayed in tissues is by staining them *in situ* (*cytochemical* method). The substance has, of course, first to be fixed in position, and the substance or grouping to be detected must react specifically with another substance to give a colour which is then observed under the microscope. Such methods have been used to detect proteins, lipoid, glycogen and ascorbic acid; also enzymes of various types, including respiratory enzymes, ribonuclease and, very recently, choline esterase (Koelle and Friedenwald, 1949). They have been applied recently to study the site of origin of various hormones in the ovaries and the placenta. Claesson and Hillarp (1947) have, by such methods, obtained evidence which suggests that the secretion of œstrogen by the ovary is accompanied by the disappearance of a steroid precursor stored in the organ, while Wislocki and Bennett (1943) have extensively studied the placenta histologically in the human and monkey and conclude, very tentatively, that gonadotrophin is produced in the cytotrophoblast and the steroid hormones in the syncytium. Similar methods are also being used to study the secretory activity of the adrenal cortex (Greep and Deane, 1947; Kar, 1947).

(3) Biological Methods

The methods so far described may not be suitable for the assay of small quantities of certain substances, but sometimes this may be done by measuring the *biological effect* which they produce, preferably in comparison with the effect produced by a known preparation of the substance under test. This method has been used very extensively in modern pharmacology and has made it possible to follow the fate in the body, and rate and mechanism of elimination, of substances like histamine, acetylcholine, some hormones and vitamins and various antibiotics.

Biological methods can be very sensitive but are also usually cumbersome and time consuming, and are therefore, as a rule, used only when other methods are not available or satisfactory. Clark (1937) has discussed the minimum concentrations of drugs which will produce effects on living tissue. A number of common drugs such as histamine and posterior pituitary extract are active at dilutions between 1 part in 10^9 and one part in 10^{12} and there is some evidence that even lower concentrations may be effective.

As Clark remarks, this is not so surprising since a solution of, for example, adrenaline at a dilution of 1 in 10^{16} will still contain 300,000 molecules per ml. An interesting example of a substance acting in very small amounts is that of the hæmopoietic factor of the liver which produces an effect in man in a dose as small as 1 μg. It will produce an effect on the growth of an organism, L. lactis, in concentrations of about 1 part in 10^{-11} (Rickes *et al.*, 1948).

One difficulty with biological effects is that they may not be very specific. For example, histamine produces a contraction of the guinea pig's intestine and tissue extracts have been assayed for their histamine content by this method. There are, however, several other substances which will produce this effect and which may be present in tissue extracts. Various methods are used to get over this difficulty. In the first place, the extract may be treated chemically by methods which will destroy other substances likely to be present, and yet have no appreciable effect on the substance to be tested. Secondly, the effect of the extract may be tested in the presence of a substance known to inhibit the active principle in a more or less specific manner. For example, if the effect is abolished by atropine it is probably due to acetylcholine or some similar active principle; and if the effect is decreased or abolished by anti-histamine substances, e.g. mepyramine, then this will support the belief that the active principle is histamine.

Another important method is to test the extract on several different tissues. For example, extracts which are believed to contain adrenaline can be assayed for their adrenaline content on the frog's heart which is stimulated, the rabbit's small intestine which is inhibited, and the cat's blood pressure which is raised. The effects produced in these various experiments can be compared with those produced by a standard solution of adrenaline. If the results obtained in the different tests are in substantial agreement, i.e. if they all show that the extract contains the same concentration of adrenaline, this will strengthen the conclusion that what was assayed in the extract was, in actual fact, adrenaline.

Biological methods of assay have been of great value in modern work with antibiotic substances. Here sensitive organisms serve as a test object and the effect on their growth of a tissue fluid or extract containing the antibiotic is compared with that of standard solutions of the antibiotic under investigation. It is only by the

use of such methods that the extensive work on the rate of absorption, metabolism and excretion of substances such as penicillin and streptomycin has been possible. These, like all biological methods, are very time consuming and attempts have been made to develop chemical methods by which such antibiotic substances could be assayed. One such method has been elaborated for streptomycin and is being investigated for the determination of this substance in tissue fluids (Boxer *et al.*, 1948).

Isotopes

This is a subject which has advanced very rapidly and the methods developed are of value in many fields of scientific investigation, not all, unfortunately, beneficial to man. The principle of these methods will first be discussed and their application to the problems of pharmacology in particular will then be considered. As will be seen the main advantages of the isotope methods are (1) that they are very sensitive and hence very high dilutions of substances introduced into the body can be detected, and (2) that the introduction of an isotope into a substance does not in any way change its chemical properties; thus the body cannot distinguish between two substances, one of which is a normal constituent and the other the same substance containing an isotope.

Physical Basis

All chemical elements have three essential constituents, namely the protons and neutrons which constitute the nucleus, and the electrons which surround the nucleus. The proton carries one positive electric charge and the electron carries a negative electric charge. The neutron carries no charge and therefore passes through an electric field without deflection. For that reason neutrons are particularly suitable for bombarding the nuclei of atoms, since they will not be deflected by charges in the atoms, and thus will be capable of getting at and hitting the nucleus. The mass of the proton and neutron is about the same, but that of the electron is much smaller, viz. about 1/2,000th that of the proton or neutron. The nucleus of an atom occupies only a very small space as compared with the whole atom: if the nucleus be regarded as a sphere about 1 cm. in diameter, then the average electron is,

in comparison, about 100 metres away, i.e. atoms are really very empty structures.

The atomic weight of an element is essentially dependent on the total number of protons and neutrons it contains. But its atomic number, and hence its chemical properties, depends on the number of peripheral electrons (carrying negative charges) which equals the number of protons in the nucleus (carrying positive charges), since the atom is electrically neutral. The atomic numbers of the elements vary from 1 to 92. It is obvious that the addition or subtraction of a neutron from an atom will not alter the charge in the nucleus or the number of electrons in the atom. Hence its chemical properties will remain unaltered even though its atomic weight is changed. It is thus possible to have two or more atoms which have the same atomic number, the same chemical properties, but different atomic weights, and these are called isotopes.

For example, an atom of ordinary hydrogen found in nature contains one proton and one electron and has an atomic number of one. Heavy hydrogen, or deuterium, contains one proton, one neutron and one electron. It therefore also has an atomic number of one, but an atomic weight of two. These two isotopes have exactly the same chemical properties and can only be separated by physical means. In actual fact the hydrogen found in nature does not entirely consist of the element with an atomic weight of one, since it contains about 0.2 per cent of heavy hydrogen. In the same way the carbon found in nature (of atomic weight 12) contains about 0.7 per cent of an isotope with an atomic weight of 13 (i.e. there is one more neutron in the nucleus), and the nitrogen in nature (atomic weight 14) contains 0.368 per cent of an isotope of atomic weight 15.

Other elements found in nature are also mixtures of isotopes. For example, chlorine owes its non-integral atomic weight (35.5) to the fact that it contains 25 per cent of an isotope with an atomic weight of 37 and 75 per cent of an isotope with an atomic weight of 35. And natural iron is a mixture of four isotopes having atomic weights respectively of 54 (6.0 per cent), 56 (91.6 per cent), 57 (2.1 per cent) and 58 (0.28 per cent).

All the isotopes so far mentioned are stable, i.e. the atoms do not spontaneously disintegrate. With the exception of heavy hydrogen they can only be identified by an apparatus called the mass spectrograph, in which the ionized atoms are accelerated in a

magnetic field, which causes them to travel in a circular path, the radius of which depends on the mass of the particular particle. A collecting plate accumulates the charges brought to it, and the relative amount of the particular isotope is determined from the value of the charge collected. Heavy hydrogen can be converted into water, and the amount present measured by means of its specific gravity. Very accurate methods have been devised for measuring the specific gravity.

Radioactive Isotopes

In some cases the constitution of an atomic nucleus is such that it no longer remains stable. The forces which hold the constituents of the nucleus together are at present being intensively studied, but it is not yet fully understood why some atoms are stable, while others must undergo decomposition. The outstanding example of an unstable element is, of course, radium, but others occur in nature, and many others have been made artificially, for various purposes, chiefly by the use of the cyclotron or in the uranium pile. These unstable elements disintegrate with emission of various types of radiation and they are therefore called radioactive. The main radiations which are emitted during the course of disintegration are :—

(1) *α-rays :* These are the nuclei of helium atoms and consist therefore of two protons and two neutrons. They have very little power of penetrating tissues and are therefore of little practical importance, though they are of experimental interest in relation to the mode of action of radiation.

(2) *β-rays :* These are negatively or positively charged electrons which have a greater power of penetration than the α-particles, but much less than γ radiation. For example, most of the β-rays emitted by radium will be absorbed by a few millimetres of tissue. The term positron is applied to a positively charged electron.

(3) *γ-rays :* These are high energy photons, i.e. quanta of electro-magnetic energy. They are exactly the same as X-rays of very short wave length. They have a high power of penetration and will easily pass through tissues and through many feet of air. They are stopped by elements of high atomic number ; thick sheets of lead are usually used as a shield against sources of γ radiation (and X-rays).

The rate at which radioactive elements disintegrate varies

tremendously. For example, radium does so very slowly while C^{11} (carbon with an atomic weight of 11) disintegrates in a matter of minutes. The half life of an element is the time required for the sample to decay to one-half of its original activity. The half life of uranium is about a billion years; of C^{14} about 5,000 years and of N^{13} less than ten minutes. Some of the radioactive isotopes that have been used in pharmacological and therapeutic work, together with their half life and the type of radiation they emit, are given in Table 14.

TABLE 14

Element	Half life	Radiations emitted
Tritium (H^3)	31 years	β
Carbon (C^{11})	205 mins.	β (positrons)
Carbon (C^{14})	4.7×10^3 years	β
Phosphorus (P^{32})	14.3 days	β
Sulphur (S^{35})	87.1 days	β
Sodium (Na^{24})	14.8 hours	β, γ
Calcium (Ca^{45})	180 days	β, γ
Strontium (Sr^{89})	55 days	β
Bromine (Br^{82})	34 hours	β, γ
Iodine (I^{130})	12.6 hours	β, γ
Iodine (I^{131})	8 days	β, γ
Manganese (Mn^{52})	21 mins. 6.5 days	β (positron), γ β (positron), γ
Iron (Fe^{55})	4 years	γ
Iron (Fe^{59})	47 days	β, γ
Arsenic (As^{73})	50 hours 90 days	β (positron) γ
Arsenic (As^{74})	16 days	β (positron), γ
Gold (Au^{198})	2.7 days	β, γ

Methods of Using Radioactive Isotopes

These substances can be used for two main purposes, namely: (1) *Experimental:* to detect substances in various tissues or fluids, and (2) *Therapeutic:* to apply radiation to certain parts of the body. Experimentally these substances have already been used extensively in various types of pharmacological research. The principle of this use is simple. A substance whose action is being investigated in the body, or on isolated tissues, is marked

or "tagged" with a radioactive isotope which acts as a "tracer" and can be detected in the body by the radiation which it emits. Since the isotopes have exactly the same chemical properties, the body is unable to differentiate between them and therefore deals with the radioactive isotope in the same way as with the normal naturally occurring isotope. The method can be so sensitive that, even if the proportion of radioactive atoms to ordinary atoms, whose course is being followed through the body, is one to several million the radioactive atoms can still be detected. The sensitivity is thus much higher than that of any chemical method. The following example, widely quoted, will illustrate this point. If a glass full of water in which all the hydrogen was radioactive were put into the sea, e.g. in the Channel, and allowed to mix with all the water present on the earth and a glassful of water were then taken, e.g. in the Indian Ocean, this would still contain many millions of radioactive atoms whose presence could be detected by the appropriate methods.

There are certain limitations to the use of this method. In the first place the isotope used must have a suitable half life : if this is too short it will have disintegrated before the experiment is finished. In experiments with isotopes of comparatively short life, e.g. radio sodium, the experiment must be started soon after the preparation of the material. On the other hand the half life must not be too long, otherwise, in many cases, the amount of activity emitted in a reasonable time is too small for measurement at high dilutions. It is also important to use these radioactive substances in small amounts since the radiations themselves might produce effects on the tissues and thus interfere with the experiment. This is particularly so when the investigation is done in man where the radiations may also produce effects deleterious to health. Elements which have a long half life are more likely to produce damage, particularly if they are deposited in tissues ; radio strontium, for example, is deposited in bone (like calcium) and will, when given to mice, cause the development of osteosarcoma at the site of storage. Radio iodine too has to be given with great care since a high proportion is taken up by the thyroid gland and may there produce damage. For therapeutic purposes higher doses can, of course, be given.

Detection of Radiation

The radio isotopes can be detected by a variety of methods, which all depend on the fact that the radiations emitted by them (α, β and γ) produce ionizations. As has been stated above, atoms consist of a positively charged nucleus surrounded by a constellation of negative electrons, the whole being electrically neutral. Radiations, in their passage through matter, eject electrons from the atoms through which they pass. An atom so ionized is left positively charged and is referred to as an ion. The electron which is ejected from an atom in the process of ionization eventually becomes attached to another atom and makes it a negatively charged ion. Both the positively and negatively charged ions, which are usually called an ion pair, are equally significant in the physical measurement of ionization. It is by the production of ionization that the radiations we have considered produce all their biological and toxic effects.

The main method of measuring radiation is by the use of Geiger-Müller counters. These have a chamber containing gaseous media in which the radiation produces ionization, and the ionization facilitates the passage of an electric current through the chamber. By means of a counting device the number of ionizations which occur in a given time can be counted. The book by Kamen (1947) should be consulted for further details of this and other methods of estimating the radioactivity of different materials. The amount of ionization which occurs in a given space can also be measured by certain types of electroscopes : in this method the ionization gradually discharges the charged leaf or thread of the electroscope.

The radiation emitted can be measured not only in body tissues and fluids but also in the living animal and in man, and this method has actually been used for determining the distribution of certain substances in man, in health and disease. The counter is applied to a particular part of the body and the radiation emitted from that part of the body is thereby determined. In most cases it is γ radiation that is counted, since β radiation can only pass through a few millimetres of tissue. For example, the counter can be applied to the region of the thyroid gland : radio iodine is then administered to the patient, and as it collects in the thyroid, it is measured by the counting apparatus. The rate at which iodine is taken up by the thyroid can thus be determined both in the normal subject and in abnormalities of the thyroid.

For the localization of radioactive material in tissues another method is being developed and is yielding very interesting results. A section of the material is placed in close apposition to a photographic plate and the β-rays emitted by the material produce development of the photoactive substance in the plate. The picture so obtained, called the *autoradiograph*, shows the distribution of the radioactive material in the tissue. In some methods the section of tissue can then be stained and since sections and photographic emulsion remain in apposition they can be observed under the microscope and the histological distribution of the radioactive substances can be seen. For example Fig. 10 (p. 122) shows the distribution of radioactive iodine in the thyroid of animals to which iodine 131 had been previously administered. It will be seen that the iodine has been taken up by the thyroid and is now localized in the colloid of the gland. Similar methods have been used for localizing radioactive phosphorus (P_{32}) in bones, etc. Methods for producing autoradiographs are discussed by MacDonald *et al.* (1948).

Radiation Hazards

It has been known for a long time that radiations produced artificially (X-rays) or emitted from naturally occurring radioactive substances, e.g. radium, are not only of diagnostic and therapeutic value, but can also produce serious damage to the body, as was indeed found out by the pioneers with these new tools. The same effects can be produced by artificially produced radioactive substances and as these are being increasingly made, and used for experimental and therapeutic purposes, this is becoming a matter of considerable importance. These radiations produce their effects by ionization and much work has recently been done on the mechanism by which ionizing radiations act on living tissues, and on the changes which may thereby be produced. The question is discussed in the book by Lea (1946).

Ionizing radiations probably produce their *biological effects* by two main primary mechanisms, namely (1) By a direct action on an organic molecule which is a constituent of the tissues. It is known that when an atom is ionized, the molecule of which it is part usually undergoes chemical change, and this may produce an important effect on the tissue of which this molecule forms a part. For example, an ionization occurring in the nucleus of a cell may

produce a chemical change in a molecule which forms part of the structure of a chromosome and this may lead to the breaking of this chromosome and hence possibly to a genetic effect. (2) The radiation may produce ionization of water, leading to the production of hydrogen and hydroxyl radicles. It is thought that the hydroxyl radicle is the more reactive of the two and may act on other molecules producing changes in them.

Whatever the primary mechanism of their action it is clear that ionizing radiations can destroy *enzymes*. This can be demonstrated not only *in vitro*, where radiations are highly effective in destroying enzymes, provided these are present in the solution in a high dilution, but also *in vivo*. It has thus been found that comparatively small doses of radiation will affect certain enzymatic processes in the living animal. For example, a dose of 500r. will produce a definite effect on glycolysis. There can be no doubt that these effects on enzymes must be an important factor in the action of radiations on living tissues.

Radiations can also destroy other *large protein molecules*, notably viruses. This includes crystalline viruses, such as tobacco necrotic virus, which are nucleoproteins and are responsible for various diseases in plants, and also viruses responsible for disease in animals, e.g. vaccinia virus. These effects are of great interest since nucleoproteins are constituents of living cells and the chromosome threads which control the genetic constitution of the cell are made up of nucleoproteins. Indeed the genes which are located on the chromosomes have properties very similar to viruses, since both are capable of producing replicas of themselves from the material in the surrounding medium, the genes doing this in the process of mitosis, and the viruses during their reproduction in an organism which they have infected. The view has indeed been expressed that viruses are "naked" genes.

Actions of Radiations on the Nucleus

Radiations produce a number of actions on the nucleus, some of which must be exerted on the chromosomes and genes, while others may affect other constituents of the nucleus. Thus radiation will delay cell division, especially when applied just before the beginning of division, i.e. just before the beginning of prophase. Unless a lethal action has been produced the cell gradually recovers from this effect of radiation. This effect may well be due to a

destruction of some enzyme constituents of the nucleus necessary for the division of the cell, and the period of recovery may represent the time needed for the enzymes to be formed again.

Several kinds of structural changes in the chromosomes can be produced by radiations. Usually breaks in the chromosomes occur at an early stage, and there is much evidence that a single ionization, occurring in, or in the immediate vicinity of, a chromosome is responsible for the ensuing break. If the effect of radiation is very marked the cell will die, but with small doses the chromosomes will unite again, either restoring the normal pattern, or producing abnormal patterns resulting from the joining together in various fashions of the several broken ends present in a nucleus in which two or more chromosome breaks have occurred.

Radiations may also produce gene mutations of the same type as have already been described in the chapter on mutagenic substances. It is not known to what extent such effects may occur in man and affect subsequent generations, but the experimental evidence suggests that they represent a definite danger. The question has been discussed recently (see report of Conference held at the British Institute of Radiology, 1947).

In addition radiation can induce lethal effects in the nucleus which are only seen in the offspring. These are not mutations and are called dominant lethals. Thus when sperms are irradiated a proportion of the eggs fertilized by the sperm fail to reach the adult stage, due to the induction of dominant lethality in the irradiated sperm.

Effects of Radiations in Man

The actual signs and symptoms of deleterious actions produced by radio isotopes are the same as those which may follow the application of radiation by means of X-rays or radium. And these changes, which affect the haemopoetic system, the gonads, the general health, and may result in local effects such as burns and in the development of malignant disease, have been extensively investigated and discussed. The effects produced with isotopes will of course depend on their distribution and localization in the body, storage and rate of elimination. As with X-rays, the repeated application of small doses is particularly fraught with danger and may lead to the development of sterility, of serious blood changes, and to malignant disease. Recent experimental

work, done to some extent as a result of the development of atomic projects, has indeed suggested that the doses capable of producing deleterious effects when applied over long periods are smaller than was previously thought. The upper tolerance limit may be as low as 0.01r. per day (see Howarth, 1948, for a short review of literature on radiation hazards), though it is not known whether some of the effects produced by such low doses are in actual fact deleterious to the body. The carefully recorded data of Dickie and Hempelmann (1947) on the morphological changes found in the lymphocytes of persons exposed to low doses of ionizing radiation are interesting in this connection. There is, it must be emphasized, no lower limit to the dose capable of producing mutagenic effects, since the effects of radiations on the genic material are additive.

Isotopes which become localized in a particular part of the body are particularly dangerous, especially if they have a long half life, since even with very small quantities a marked and prolonged effect is produced in their immediate vicinity. This is seen experimentally with radium and also with strontium 89 (half life=55 days) which, like radium, tends to be concentrated in the bones where it frequently leads to the development of osteosarcomata. Similarly the subcutaneous injection of as little as 1 μg. of plutonium may cause the production of a fibrosarcoma at the site of injection (Brues *et al.*, 1947).

Applications of Radio Isotopes

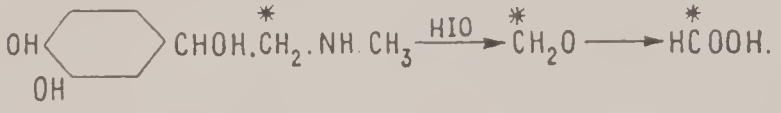
Some of these, experimental and therapeutic, are dealt with in other chapters, e.g. the use of radio iodine in Chap. 6 on the thyroid and that of radio iron in Chap. 13 on the blood. A number of other uses will be described, not in an attempt to cover the subject exhaustively, but merely to illustrate the various ways in which these new substances have proved of value in various types of pharmacological investigations and in therapy.

The Biological Synthesis of Adrenaline

There is indirect evidence that phenyl alanine may be converted to adrenaline by surviving slices of adrenal medulla (Devine, 1940), but there is no direct evidence that such a change can occur in the body. By the use of radioactive elements this conversion has been demonstrated experimentally in rats (Gurin and Delluva, 1947).

Radioactive carbon (C_{14}) was inserted into phenyl alanine and this substance was then injected into rats. After various short periods the animals were killed and the adrenal glands extracted. The adrenaline so recovered was found to be radioactive, showing that the phenyl alanine had been converted to adrenaline.

Phenyl Alanine — Adrenaline



Adrenaline — Formic Acid

The radioactive carbon is labelled*

The location of the radioactive carbon in the adrenaline was confirmed by oxidizing the substance with periodic acid and obtaining formic acid containing practically all the radioactivity. Similar results were also obtained when the phenyl alanine was labelled with tritium (H_3).

It is obvious that similar methods could be used to study the fate of adrenaline and other amines in the body.

The Formation of Antibody and its Relation to Antigen

There is evidence that the reticulo-endothelial system is concerned in the formation of antibody and this has now received support from experiments involving the use of radioactive phosphorus (Libby and Madison, 1947). A pure protein antigen was obtained by using tobacco mosaic virus, which can be crystallized and into which the radioactive substance was incorporated by the ingenious method devised by Born *et al.* (1941) and by Stanley (1942): the tobacco plants are inoculated with the virus and grown in bottles in a nutrient solution containing sodium hydrogen phosphate in which radioactive phosphorus has been incorporated ($Na_2HP^*O_4$). The level of radioactivity in the nutrient solution is maintained by fresh $Na_2HP^*O_4$ for 30 days during which the plants are allowed to grow, and the radio phosphorus is thus incorporated in the nucleic acid part of the

nucleoprotein. The plants are then extracted for tobacco mosaic virus. The virus is recrystallized several times and evidence that the radioactivity is associated with the virus molecule is afforded by the finding that the specific activity remains constant during repeated crystallizations.

The same principle has been used to incorporate radio active carbon 14 into drugs derived from plants. So far digitalis purpurea and nicotiana rustica have been used. They have been grown in an atmosphere containing radioactive carbon dioxide. Pure digitoxin

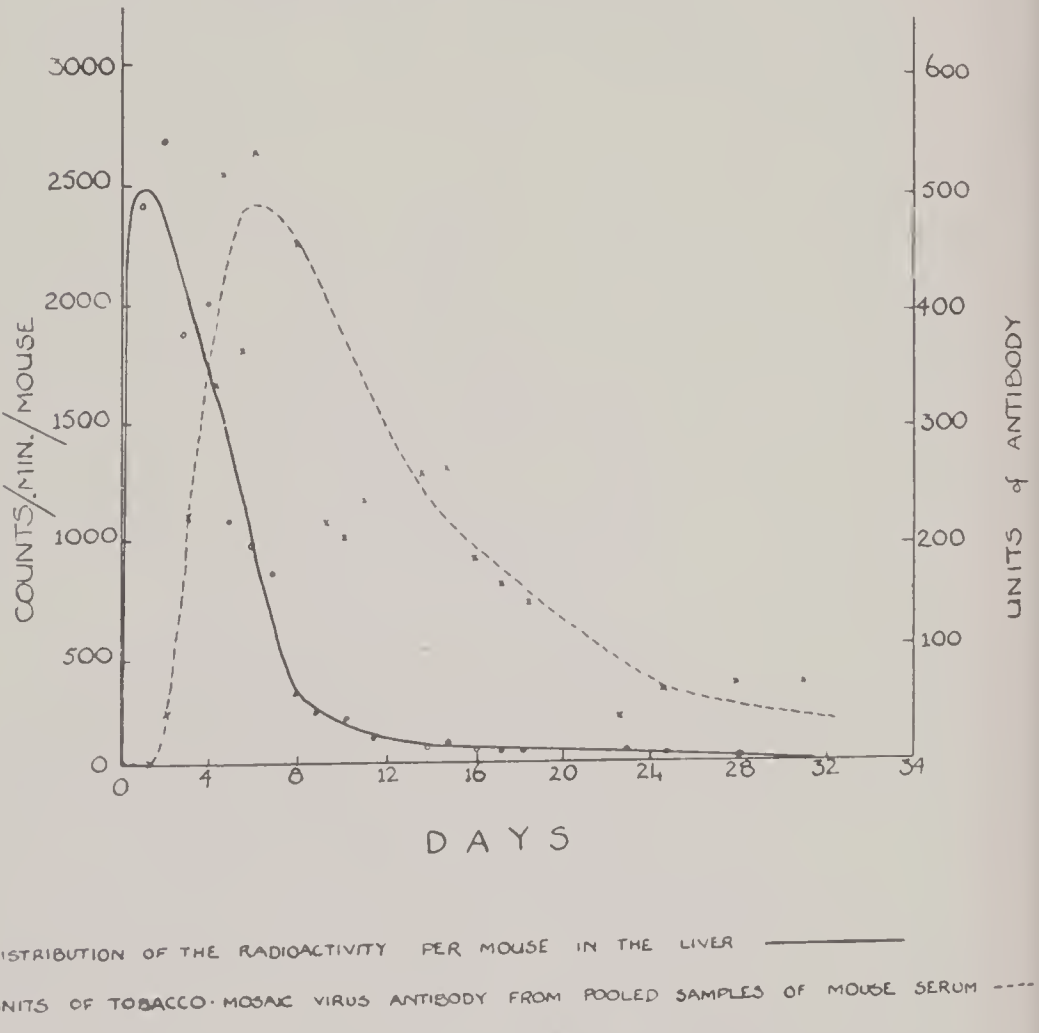


FIG. 43

Showing the relation between the presence of antigen (radioactive tobacco mosaic virus) in the liver of the mouse, and the formation of antibody.

(After Libby and Madison, 1947.)

and nicotine respectively were then extracted from the plants and found to be radioactive, evidently due to the incorporation of the radioactive carbon, first into the plant and then into the drug produced by the plant. Similar experiments are being performed with digitalis lanata, papaver somniferum and atropa belladonna (Geiling *et al.*, 1948). Such drugs should prove very interesting for pharmacological investigations.

The radioactive tobacco mosaic virus containing radioactive phosphorus was injected intravenously into mice, and its distribution determined both by taking radioautographs of sagittal sections of the animals, and by determining the specific activity of weighed samples of various organs which had been dried and incinerated. It was found 24 hours after the injection that about 2/3rd of the material was located in the liver and about 3 per cent in the spleen. If sodium phosphate ($N_2HP^*O_4$) is injected into mice, the activity is fairly uniformly distributed throughout the animal, confirming that it is the tobacco mosaic virus, and not radio phosphorus, which is specifically taken up by the liver. Some 14 days after the injection of the radioactive tobacco mosaic virus most of the activity in the liver and spleen had disappeared, and the authors calculate that the half life of the injected virus is about two days. Antibody formation to the injected antigen was also determined. It reached its maximum at the 6th to 7th day after injection, and then gradually decreased. These results suggest that the liver plays a major rôle in the removal, destruction and elimination of the foreign protein virus, and also in the production of specific antibody. The relation between the disappearance of the tobacco mosaic virus from the body and the decrease in circulating antibody may indicate that antibody formation occurs only when antigen is present to stimulate it. The further investigations which these authors are undertaking ought to yield very interesting results.

Some Uses of Radio Sodium

Radio sodium has been used in a number of investigations, some of which are reviewed by Quimby (1947), while its use in the study of heart failure is discussed in the *Lancet* (1948). This element is particularly suitable for introduction into the human body since it has the short half life of 14.8 hours.

The Mechanism of Shock. Sodium, like thiocyanate, is dis-

tributed throughout the extracellular fluids of the body, i.e. throughout the plasma and lymph spaces, and studies with radio sodium have shown that this represents about 21 per cent of the body weight. There is evidence that in shock caused by burns or trauma, a redistribution of the sodium and potassium in the body occurs: presumably the barrier between the extracellular and intracellular fluids is affected and the sodium can to some extent penetrate into the intracellular fluid. If this is true it is important clinically from the point of view of treatment. To investigate whether this occurs, Fox and Keston (1945) performed experiments with radio sodium on mice. Shock was induced by inflicting standard burns, or by applying a tourniquet to both limbs, and radio sodium was then injected; it was found that the damaged tissues (skin and muscle), which normally contain little sodium, took up a large amount of the administered radio sodium. This shows that concentration of sodium occurs in the damaged parts of the body, thus depleting the rest of the body of this element, and this may be an important factor in the mechanism of shock. If this is true, then the administration of sodium to such animals in a condition of shock should be of therapeutic value, and it was indeed found that burned animals treated by intraperitoneal injection of normal saline recovered, while similarly burned untreated animals died.

The Investigation of Peripheral Vascular Disease. In certain conditions it is desirable to know what the blood supply to a limb is, whether it is reasonably normal, greater than normal, or severely restricted by the disease. Smith and Quimby (1945) have attempted to do this in the following manner: they inject a standard amount (100 μc.) of radio sodium into their subjects and then take counts from the sole of the foot. The counter, enclosed in a lead container which acts as a shield, is strapped to the sole of the foot. The lead shield has a window in it which allows the radiation from the foot to reach the counter, which then records essentially the amount of radio sodium present in a limited part of the foot. Following the intravenous injection of the material, it reaches the blood vessels in the foot and then gradually diffuses out of the vessels into the tissues. Hence the count which is low at first, gradually rises to reach a maximum in rather less than an hour. The blood supply of the part will obviously be one of the factors which control the rate of "build-up", and hence the rise

in the counting rate, of radio sodium in the foot. According to the authors the curve obtained gives an indication of the vascular supply to the limb. In normal cases these curves fall within a narrow range. Low curves, corresponding to a slow rate of diffusion of the material into the foot, are obtained in conditions where the blood supply is decreased, e.g. arterial obstruction, arteriosclerosis, vascular degeneration. High curves are found in patients where the blood supply is increased, as for example in inflammation or where the blood vessels are relaxed by sympathectomy, nerve block, or for other reasons. The results should be of prognostic value and help in deciding on the therapeutic measures required.

The authors are at present studying the effect of drugs and of physical therapy on patients with peripheral vascular disease. Their preliminary results show that histamine, given by iontophoresis or by intra-arterial injection, brings about a rise in the rate of diffusion of radio sodium (Mufson *et al.*, 1948).

There must be a number of factors, apart from the condition of the blood vessels, which control the rate at which the sodium is built up in the tissues and these obviously require further investigation. It is indeed remarkable and very satisfactory that with such a number of variables, and with this rather empirical procedure, the build up curves in normal subjects fall within such a narrow range.

The Rate of Flow of Venous Blood in the Legs. Venous thrombosis is influenced by three main factors—changes in the constitution of the blood, damage to the blood vessels, and disturbances of the circulation. The first two factors have been extensively investigated, and the use of anticoagulants in this connection is discussed in Chap. 14. There is, however, little direct evidence on the rôle of the circulation. The introduction of radioactive tracers has now made possible the direct measurement of the rate of blood-flow in a limb by introducing such a tracer at one point and determining its arrival at some other point with a suitably placed counter sensitive to the type of radiation emitted. Wright and her co-workers (1948) have used radio sodium for this purpose. A γ-ray counter, so shielded by lead as only to record rays coming from vertically below, is placed over the groin. The solution of radio sodium (as sodium chloride) is injected into a vein on the dorsum of the foot as rapidly as possible, and its arrival in the region of the groin is recorded by the counter,

which indicates an increase in the counting rate above the background. The time of arrival is usually quite clear cut ; and from a knowledge of the time of injection, the time of arrival at the groin and the length of the leg, the average rate of flow can be calculated. The average time found for normal subjects was 18 ± 0.9 sec., the observations ranging from 4 to 50 seconds. Technical details of the method used are described by Osborn and Wright (1949).

During pregnancy the rate of venous blood flow in the leg is progressively slowed ; enlargement of the fœtal head increases the slowing and a still further retardation occurs in labour (Wright, personal communication). In the puerperium the venous flow rapidly returns to normal. The relation of some of these findings to the occurrence of venous thrombosis is discussed by Wright *et al.* (1949).

The Assay of Deoxycortone. A number of methods are in use for the assay of adrenal cortical steroids but none of them are either accurate or particularly sensitive. By the use of radio sodium, Dorfman *et al.* (1947) have elaborated a method which allows of the estimation of as little as 1 μg. of deoxycortone. With methods depending on the measurement of ordinary sodium retention, the least amount of deoxycortone acetate which can be detected in dogs is of the order of 700 μg. Dorfman and his co-workers performed their experiments on adrenalectomized rats, which lose sodium from the body. Administration of cortical steroids causes a retention of sodium. The steroid to be tested is given to adrenalectomized rats, which are then injected one hour later with the radio sodium, given as sodium chloride. The animals are then placed in a metabolism chamber and the urine collected for 4–6 hours. An appreciable retention of sodium is produced by very small amounts of deoxycortone, but further work is required on the quantitative significance of the results, and on the effects of other adrenal cortical steroids.

Dorfman (1949) has also described a method for the assay of deoxycortone which depends on the use of radio potassium in the adrenalectomized male rat ; as little as 10 μg. of deoxycortone can be detected.

The Localization of Brain Tumours

Moore (1948) has used radio-iodine in the localization of brain tumours. He had found previously that such tumours concentrate

the dye, fluorescein, and also found, in experiments on mice, that diiodofluorescein is similarly concentrated by experimentally induced brain tumours, which contain up to 80 times as much dye as the surrounding brain tissue. Iodine 131, which emits γ radiation and can therefore be detected from the outside, was incorporated to make diiodofluorescein and injected intravenously in patients suspected of having brain tumours, in amounts calculated to contain 500–600 μc. of radioactivity. After 2–4 hours the dye is taken up by the tumour, if present, and counts are taken over various parts of the skull. The highest counts, due, of course, to the γ radiation, are found over the area of the tumour. The preliminary results suggest that this method may prove of value in the diagnosis and localization of intracranial tumours.

It has been found that when inorganic phosphate containing radioactive phosphorus (P_{32}) is administered to patients with brain tumours, and biopsy material then taken, the radioactivity is higher in the tumour than in the normal brain tissue. Chemical fractionation also showed a small but significant concentration of the phosphorus in the nucleoprotein of both cytoplasm and nucleus. This method is being used for the accurate localization of brain tumours but the investigation is still in its preliminary stages (Selverstone *et al.*, 1949 ; Erickson *et al.*, 1949).

The Penetration of Drugs into Cells

Much work has been done on the distribution of drugs in various parts of the body, but there is little information about the penetration and distribution of drugs in cells, although this is probably of great importance in understanding their mechanism of action. One of the difficulties has been the small amounts of material which have to be identified and located, and it is possible that the radio isotope technique, so delicate and selective, may lead to progress in this field. Drugs can be tagged by means of a suitable isotope and their fate followed by counter methods. Even radio-autography may ultimately be used although, at present, the limits of resolution with this method are scarcely sufficient to allow of the localization of drugs in cells.

One type of approach to the problem which may prove successful with drugs is best illustrated by reference to the work of Marshak (1941) on the uptake of phosphorus and its incorporation into various parts of normal and tumour cells. The phosphorus was

administered as radioactive P_{32} incorporated in $Na_2HP^*O_4$, of which a solution was injected intravenously into rats and mice. The mice had lymphomata and the rats sarcomata. At intervals after the injection tissues were removed, freed from blood, and the nuclei and cytoplasm of the cells separated by a special procedure involving treatment with citric acid. The nuclear material was further fractionated by various chemical methods in order to determine into which constituents the radio phosphorus was incorporated ; the nucleoproteins were, of course, of particular interest. It was found that phosphorus is rapidly taken up by cell nuclei and that the nuclei of tumour cells accumulate more than those of normal cells. This is merely due to mitotic activity and not to a form of metabolism peculiar to tumour cells. A large percentage of the nuclear phosphorus is present in the nucleoproteins, up to 90–95 per cent in the case of lymphoma nuclei. There are a number of other interesting observations, e.g. that synthesis of nucleoprotein occurs even in nuclei of cells which are not undergoing mitosis, but the original paper should be consulted by those who are specially interested.

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CHAPTER 17

EVALUATION OF THE ACTIONS OF DRUGS IN MAN

In this chapter it is proposed to discuss the problems which arise in the course of therapeutic trials in patients, and to describe some of the statistical and other procedures required for evaluating the actions of the drugs used.

It is of course common knowledge that the actions of drugs in man may differ from those in animals, not a surprising occurrence when one takes account of the considerable variations in response to drugs which may exist among different species of experimental animals. In some instances it is possible to obtain valuable information by studies of drug action on normal persons. A number of such examples have already been mentioned in other chapters, for example, the effects of analgesics on pain threshold, the actions of anti-histamine drugs on histamine wheals and flares in normal skin, and the production of skeletal muscular paralysis by curare-like drugs. This type of investigation is useful not only to reveal the probable therapeutic value of drugs, but also to give a preliminary idea of their side-effects. However, there are many pathological states in which drugs are effective through actions which cannot be studied in normal man. For example, the actions of digitalis preparations in normal subjects give no hint of their effectiveness in congestive heart failure; the assay of liver preparations by the reticulocyte response can only be measured in patients with pernicious anaemia, and the effectiveness of chemotherapeutic remedies in man can only be studied in the presence of the appropriate infections. In all these instances accurate observations are required in patients to determine the therapeutic value of the drug under investigation.

Therapeutic Trials in Man

The actions of drugs on the course of human disease are of such importance as to justify a full discussion of the methods by which they may be studied. The problem concerns not only the evalua-

tion of new drugs but also a proper estimate of the value of drugs which have long been used. The aim of drug therapy is simply enough to exert a favourable influence on the course of disease, but it is at once obvious that some drugs fulfil this purpose much more effectively than others. For example bacterial chemotherapeutic agents such as the sulphonamides and penicillin, which act by virtue of a highly selective action on the causative agents in certain infections, produce what may be correctly termed a cure, whereas in many other instances, e.g. the use of analgesics to relieve pain, or the administration of digitalis to treat heart failure, the action is either symptomatic or palliative, with little or no influence on the course of the underlying pathological change.

Drugs and the Natural Course of Disease

Whatever the technique of assessment of drug action, it is always essential to consider the background against which the therapeutic effect is to be measured. We must know therefore the natural course of the disease, either when untreated or when treated by previous methods. Now the course of some diseases is very brief and fairly consistent, whereas in other diseases it is slow and sometimes intermittent. Thus on the one hand, in pneumococcal pneumonia, streptococcal septicæmia or meningococcal meningitis, we are dealing with conditions which run a speedy and fairly regular course, and, whatever the effect of treatment may be, the end result can be rapidly and surely measured. On the other hand, in slowly progressive diseases like disseminated sclerosis and Hodgkin's disease, not only is the time scale greatly enlarged, but natural remissions, or at least halts in the progress of the disease, may complicate the evaluation of treatment. Many mistakes in therapeutic assessment have arisen from failure to take account of such points.

Effect on Mortality Rate. In diseases which have a high natural mortality rate the simplest measure of therapeutic success is of course a reduction of this rate. One of the most striking instances of such an effect is the case of subacute bacterial endocarditis in which the untreated mortality rate of close on 100 per cent has been reduced by penicillin to somewhere about 25 per cent in all cases, and to about 10 per cent mortality from the infection *per se*. Similarly the effect of streptomycin in tuberculous meningitis has

been to reduce a 100 per cent mortality rate to about 65 per cent. Even in chronic diseases like pernicious anæmia, with its remissions, the reduction in mortality rate by liver therapy is one of the simplest measures of success; indeed in this case the most striking testimony to the value of treatment is the fact that the patients usually die from some other disease!

Other Measures of Drug Action. However, in diseases with an extremely low mortality rate (and even in those with a high mortality rate), other measures of drug influence are available. In the case of the infections already mentioned, the rapid reduction of body temperature, the disappearance of symptoms and signs, and the obvious shortening of the course of the disease will be taken into account. In other cases it is not so much the immediate relief of presenting symptoms and signs which is desired, but, as in the treatment of syphilis, the prevention of ultimate complications. Much the same could perhaps be said of the use of salicylates in rheumatic fever, except that their value in preventing the development of heart disease is much more problematical than the actions of antisyphilitic drugs in syphilis.

Even in long established diseases, effective treatment may rapidly restore normal function. For example the actions of thyroid in myxœdema, antithyroid drugs in thyrotoxicosis, liver extract in pernicious anæmia, and to take a very recent case, cortisone (Kendall's Compound E) in rheumatoid arthritis, all show how chronic pathological and functional changes may be speedily reversed and the effects of treatment easily observed.

Drug Toxicity. So far we have considered this question solely from the aspect of therapeutic benefit, but we have also to take into account the possibility that drugs may sometimes be harmful. A drug may produce a desired therapeutic action but if this occurs only with doses which exert marked side-effects it is usually of no practical value. With all drugs a wide margin between the therapeutic and toxic ranges is desirable; this factor may be expressed as the "therapeutic ratio" which is defined as "the minimum dose which produces toxic effects," divided by "the dose which produces a full therapeutic effect." Drugs like digitalis and salicylates, particularly the latter, have a very small ratio; indeed the ranges may overlap so that full therapeutic doses of salicylates in rheumatic fever almost inevitably lead to side-actions such as nausea, deafness and tinnitus. Recent reports by

Meyer and Ragan (1948) and by Camelin *et al.* (1949) suggest that sodium gentisate, in doses which produce practically no side-actions, is therapeutically as effective as sodium salicylate in this disease ; in other words it has a higher therapeutic ratio. The greatest margin of safety is seen with penicillin, of which it is possible to administer say a hundred times the normal therapeutic dose without producing any toxic effects (apart from allergic hypersensitivity reactions).

The toxic actions produced by drugs are of various kinds and many of those seen in man cannot be anticipated from animal studies. Acute toxic symptoms, e.g. vomiting and giddiness, are soon detected ; allergic hypersensitivity reactions of the classical type, e.g. skin eruptions and drug fever, and possible allergic reactions such as agranulocytosis, thrombocytopænia and liver necrosis, will naturally take longer to be revealed. With some toxic effects, such as peripheral neuritis, or a liability to produce addiction, the frequency can only be fairly assessed after months or years of observation.

It is thus clear that in the final evaluation of any drug therapy it will take some years before the true benefits, together with the drawbacks, can be fairly judged in relation to the drug's influence on the natural course of disease.

Methods of Assessment of Drug Action

It is very tempting to allow the assessment of the therapeutic value of a drug to be based solely on the subjective impressions of the observer. Now no one could deny that a final judgment on the question will take account of factors which may be difficult to measure, but it is most important that, where possible, objective measures of response should be used. Records of body temperature, respiration rate, heart rate, blood pressure, body weight, urinary output, changes in physical signs, etc., are simply made at the bedside, though in other cases more elaborate procedures such as blood counts or bacteriological and biochemical investigations will be required.

Studies on Selected Cases

After the introduction of a new drug, while it is still in short supply, it is desirable to seek an early assessment of its value by careful observations on a small number of cases. Recent

illustrations of this procedure are provided by Heneh's studies on the action of cortisone in rheumatoid arthritis, and by records of the action of chloromycetin (chloramphenicol) in typhoid fever. It should, however, be pointed out (*a*) that such preliminary observations are neeessarily based on small numbers of patients and may, therefore, not be truly representative, (*b*) that it is assumed that the natural course of the disease is well enough known for any modifieations of this course to be at onee obvious and (*c*) that the long term effeets of treatment or the onset of delayed toxie actions will not be revealed by this method.

The Patient as His Own Control

One proeedure which may be adopted in studying the effects of drugs in chronic diseases is to make the patient serve as his own control by first allowing him to remain untreated for a reasonable period of time, during which observations of his eondition may be made. This method has been used for example in the assay of liver extraets in pernieious anæmia, the evaluation of digitalis preparations and organic mercurial diuretics in heart failure, the study of antithyroid drugs in thyrotoxieosis, and the assessment of anti-histamine drugs in allergic states. When improvement has oeeurred, apparently due to the drug, the effeet of withholding the drug ean then be observed.

A very useful development of this technique allows the comparison of the effectiveness of different drugs with a given action. The method has been used by Gold *et al.* (1941) in standardizing digitalis preparations in patients with auricular fibrillation, and by Hewer *et al.* (1949) in the comparison of analgesie drugs in individual patients, but the best illustration is provided by the observations of Bain *et al.* (1949) on the comparative effectiveness of mepyramine and promethazine in the treatment of 20 patients with chronic urticaria. Before treatment all the patients had moderate to severe urticaria. Mepyramine was then given orally in increasing daily dosage until the urticaria was controlled, or until side-effeets prevented its further administration. The same 20 patients were then given promethazine in the same way. This proeedure, which corresponds to what has been called a "Crossover Test" in biological standardization (e.g. insulin assay in rabbits) revealed that promethazine, given in one nightly dose of 25 mg., was as potent as 360 mg. of mepyramine, given in a dose of 120

mg. t.d.s. (i.e. promethazine was 14 times as potent as mepyramine). More important was the demonstration that the urticaria preventing dose of promethazine produced fewer side effects than the corresponding dose of mepyramine, so that 14 of the 20 patients preferred the former to the latter. Such a test provides much more useful information than many hundreds of haphazardly collected observations.

Now a patient can act as his own control not only in time, but also in space. For example in simultaneously developing, symmetrically distributed lesions, involving say the eyes or skin, it is possible to assess the value of local applications to one side by comparing the responses of these treated areas with those of the untreated areas on the other side of the body. This technique has been used, for example, to compare the efficacy of local applications used in the treatment of burns.

Comparison of Present Results with Previous Statistics

In some diseases previous statistics may be available as a background for the evaluation of a new drug. For example Colebrook and Kenny (1936), in their studies on the effectiveness of prontosil rubrum and sulphanilamide in puerperal sepsis, compared the results obtained after these drugs with figures obtained in previous years without chemotherapy. The effects of sulphonamides in pneumonia and meningococcal meningitis, and the effects of penicillin in staphylococcal infections and in venereal diseases were all assessed by comparing the results recorded after administration of these drugs with those previously obtained. When a natural mortality rate of 25 per cent is reduced to 10 per cent (e.g. sulphonamides in pneumonia) and there are many thousands of individuals in each group, the difference is so large as to need only the simplest statistical analysis to show its significance.

There is, however, one difficulty associated with this method of comparison. It is well-known that the virulence of certain infections varies from year to year, so it is always possible that during the period of observation of the actions of a new drug, the background may have changed from previous years, thus vitiating the evaluation of the treatment.

Simultaneous Controls

In most therapeutic trials the best procedure is to make

observations on treated patients and on controls at the same time, using some method of random selection, as will be described later. Such tests overcome the difficulty, discussed in the previous paragraph, which might arise from temporal fluctuations in the natural course of the disease. The control patients will either receive no treatment at all, or they will receive some drug previously used for the disease; in certain instances, to take account of psychological influences, pharmacologically inert substances such as lactose are administered in a preparation indistinguishable from that of the active drug under test.

There is one really important question which may arise with this procedure. It is a moral one. If, early in the test, it appears that the new drug is more effective than the control drug, or if, in cases where the controls receive no treatment, the drug strikingly alters the course of the disease, when should the trial be terminated? This is naturally a difficult question to answer dogmatically, but whilst of course no one would wish to deprive patients of the best treatment available, it is probably true to say that a properly designed therapeutic test will reveal the benefits to be derived from a new treatment more quickly than even large numbers of haphazard observations. In practice the dramatically effective drugs will be revealed in preliminary trials on small numbers of patients, so that subsequent more extensive tests will be approached with this knowledge in mind. It is clear that the simultaneous control technique is best suited to the trials of drugs whose effects may be definite, but not outstanding, and two illustrations of this type of action will be fully discussed later on.

Psychological Factors

The actions of some drugs, such as digitalis or penicillin, are probably not influenced at all by suggestion, but in therapeutic tests of drugs whose actions are exerted wholly, or in part, on the central nervous system, or which are to be administered to patients whose diseases exhibit a marked psychological component, the results may be difficult to assess. Drugs such as analgesics or hypnotics work in a complicated manner, by no means wholly dependent on what we should describe as purely pharmacological actions, and the symptoms and signs of diseases such as peptic ulcer, ulcerative colitis, asthma and angina pectoris may be readily influenced by a number of apparently non-specific agents.

Attempts to assess the part played by suggestion have been made by the administration of dummy tablets (containing, for example, lactose) or by injections of sterile saline in such a way that the patient is unaware whether he is receiving an inert or a pharmacologically active preparation. Indeed it is best that he should not be told of the possible alternatives. Such inert preparations have shown that in up to 50 per cent of administrations of analgesic drugs the relief of pain is independent of any pharmacological action (Asher, 1948). Gill (1948) has shown how patients with peptic ulcer may show not only relief of symptoms, but also healing of the ulcer, after injections of sterile water.

Now it is perfectly right that the factor of suggestion should be made full use of in therapeutics, and the good doctor does so, but we do feel that some distinction should be made between pharmacological and psychogenic actions, if for no other reason than that the former may thereby be more effectively reinforced by the latter.

Exclusion of Other Factors

In studying the influence of any drug upon the course of a disease, it is important to take account of the effects of other procedures which may be simultaneously used in the treatment of the patient. For example, the beneficial effects of rest in bed on patients with heart disease, thyrotoxicosis, peptic ulcer or pulmonary tuberculosis, or the improvements which may follow appropriate diets in such patients, must all be assessed in attempting to define the influence of any drugs used on the course of events.

Finally, in considering the influence of any one drug on the course of a disease, it may be necessary to eliminate all other drug therapy, in other words to test one remedy at a time. In some instances, two drugs whose different actions converge towards the same end, may be studied in combination, e.g. sulphonamides and penicillin in pneumonia, organic mercurial diuretics and acidifying agents in congestive heart failure, or streptomycin and p-aminosalicylic acid in pulmonary tuberculosis. In all cases the purpose of a therapeutic trial of any drug is to determine what influence the drug has on the course of the disease which is being studied.

We have discussed some of the more important factors relating to therapeutic trials in man, and we have seen that various

procedures may have to be used according to the nature of the disease, and the effectiveness of the drug under investigation. Where drug actions are very striking only the simplest of statistical procedures are required, but in other cases more elaborate statistical planning and analysis are essential for speedy and economical evaluation, and in the following section a simple outline of some of the procedures employed will be presented.

Statistical Methods in Therapeutic Trials

The need for statistical methods in therapeutic trials arises largely from the variability in response from one individual patient to another, and the aim is to find some approximate mathematical expression which will indicate the most probable response of all patients with a given disease or symptom, when treated with a given drug. It is most important to realize that statistical methods are as much required in the planning of the trial as in the analysis of the results, and it is useless to expect a statistician to extract significant answers from even accurate data collected without adequate forethought and organisation. Finally, no amount of statistical effort can compensate for inaccurate measurement or careless observation.

The following brief exposition of statistics in relation to therapeutic trials is designed firstly to summarize some essential points in the approach to the problem, and secondly to discuss fully, by way of illustration, two recent important papers. The following are some of the books which have been consulted in preparing this section and readers are strongly advised to refer to them for details which may not be clear from this short account.

The Principles of Medical Statistics by A. Bradford Hill (4th Ed. 1948); this excellent book is designed to explain statistics to the non-mathematical medical man, and is the source of much of what follows; An Introduction to the Theory of Statistics by G. Udney Yule and M. G. Kendall (1946) is an admirable discussion for more mathematically minded readers; two books by R. A. Fisher, Statistical Methods for Research Workers (10th Ed. 1946) and The Design of Experiments (1947) are classical masterpieces, but definitely difficult for all but the mathematician.

The first problem concerns the *presentation of statistics*. As Bradford Hill has emphasized, the fundamental raw data must be presented in full in the form of *Tables*. The construction of these

tables is designed to reveal the *Frequency Distribution* of some event, e.g. the incidence of death or survival after administration of different doses of a drug in the treatment of an infection. On the basis of these data *Graphs* may be drawn to reveal the trend of events to a rapid glance from the expert, or to summarize the whole situation for the statistically resistant reader; it must, however, be stressed that graphs give less information than tables and are no substitute for them. The tabulated material is often presented graphically in the form of a *Frequency Polygon*, or more accurately as a *Histogram*. Table 15 is designed to illustrate the technique of grouped tabulation of data. The figures are taken

TABLE 15

Dose of Sodium Salicylate	Number of Individuals who developed toxic symptoms	
0–20 grains	1	
20–	2	
40–	8	
60–	14	Mean Toxic Dose = 135.1 grains
80–	18	Median ,, ,, = 130 ,,
100–	38	
120–	26	Standard Deviation
140–	27	= 52.2 grains
160–	19	Coefft. of Variation
180–	19	= 38.7%
200–	4	Standard Error of Mean
220–	2	= 3.82
240–	4	
260–	1	
280–300	4	
	Total 187	

The grouped data in this table are taken from a paper by Hanzlik (1913) and show the number of Females who developed toxic symptoms after different doses of orally administered sodium salicylate (used in the treatment of rheumatic fever). Inset: Some statistical measures of these data.

from some old work by Hanzlik (1913) on individual variations in sensitivity to the toxic effects of sodium salicylate given by mouth to patients with rheumatic fever. The data here presented concern only *females*, and show the number of individuals who developed toxic symptoms after different doses of the drug when it was given hourly (10–20 grains in each dose). Fig. 44 shows a frequency polygon, and Fig. 45 a histogram of the data in the table.

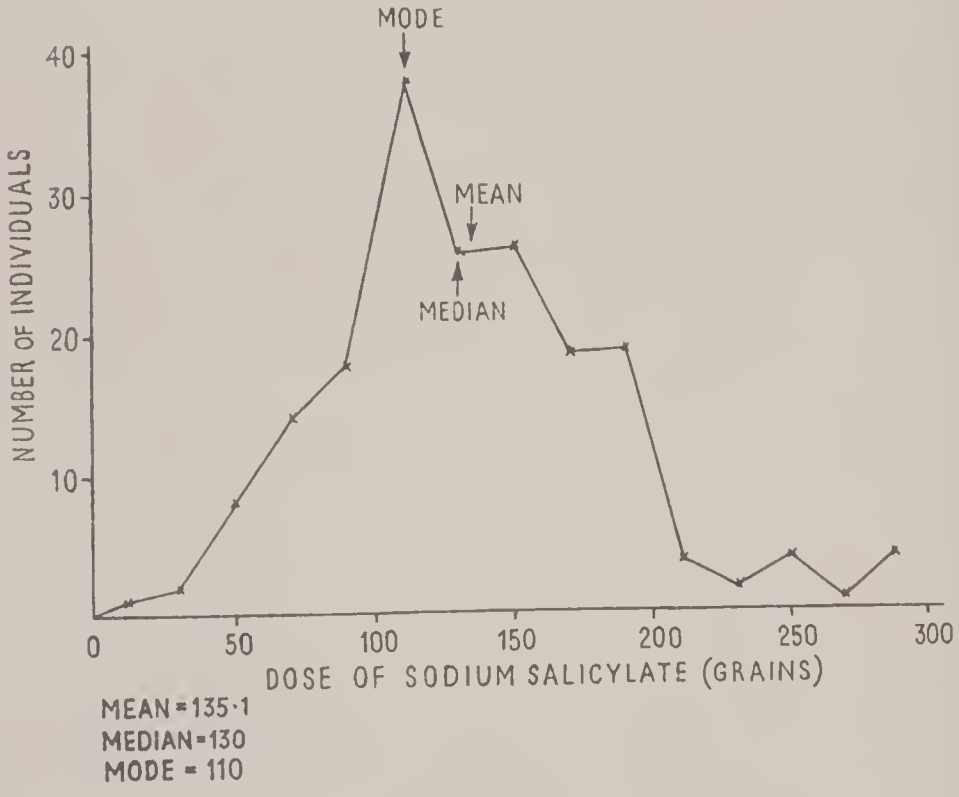

FIG. 44

A frequency polygon of the data set out in Table 15.

Now, when the statistical data have been arranged in order as just described, the next problem is to find some relatively simple mathematical expression to describe the characteristics of the distribution. This is of course particularly necessary when it is desired to compare one set of observations with another, as for example in comparing the therapeutic effectiveness of two different drugs. The most important characteristics of any frequency distribution are :—

(1) The **Central Tendency** of the observations (Dawson, 1933).
(2) The **Degree of Scatter** or **Dispersion** of the observations.

(1) Much the most commonly used measure of the central tendency of the observations is the *Arithmetic Mean* or *Average*, which is the sum of all the observations divided by the number of observations. Other measures used are the *Median* and the *Mode*. The *median* of a series of observations is the value of the central or middle observation when all the observations are arranged in order of magnitude, i.e. half the observations fall below and half lie above it. This value is much used in experimental pharmacological work especially in measurements of drug toxicity where

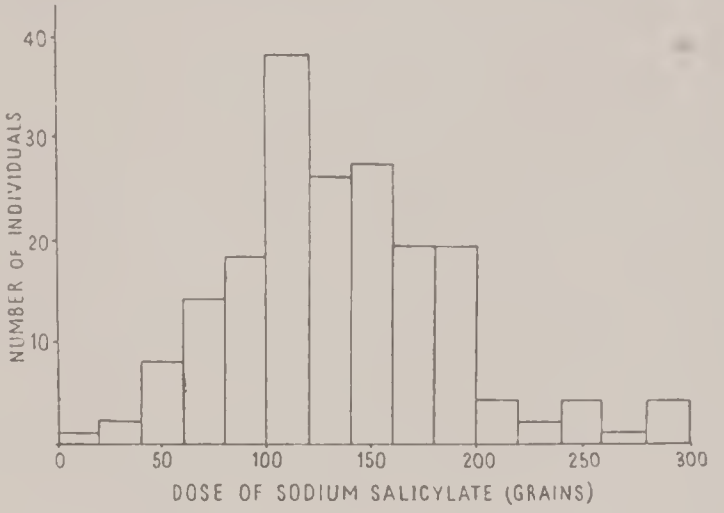

FIG. 45

A histogram of the data set out in Table 15.

the expression L.D.50 refers to the Median Lethal Dose. The *mode* is the most frequently occurring, or most "fashionable," observation in a series; this expression is much less used than the mean or median. To illustrate these measures from the data of Table 15, it is found that the mean value of the toxic dose of sodium salicylate is 135.1 grains, the median is 130 grains, and the mode is 110 grains (Fig. 44).

(2) The degree of *scatter*, *dispersion* or *variability* of the observations must clearly be taken into account in any frequency distribution. The *range*, i.e. the distance between the lowest and highest values of the observations, is the simplest measure of scatter but is usually of little value since it gives no estimate of the

distribution of the majority of observations within that range. Other measures of scatter include : (*a*) the *mean deviation* which is obtained by adding together (ignoring the sign + or −) the deviations or differences of all the observations from the mean, and dividing by the number of observations ; (*b*) the *variance* which is the sum of the squares of the deviations divided by the number of observations, and most important of all (*c*) the *standard deviation* which is the square root of the varianee. This is usually expressed in symbols as follows :—

$$\sigma = \sqrt{\frac{\Sigma\, d^2}{n}}$$

where σ = Standard Deviation
$\Sigma\, d^2$ = the sum of the squared deviations from the mean and n = the number of observations.

$$\text{The varianee} = \frac{\Sigma\, d^2}{n}$$

For the actual method of calculation of the Standard Deviation Bradford Hill's Principles of Medical Statistics should be consulted.

A large standard deviation means that the frequency distribution is widely scattered about the mean. In eomparing the standard deviation of one frequency distribution with that of another it is important to appreciate that the standard deviation is expressed in the same units of measurement as the original observations. In order to compare the degrees of variability in frequency distributions of different variables measured in different units the *Coefficient of Variation* is used ; this is $\frac{\sigma}{m} \times 100$ (where m = the mean). This is clearly independent of the units of measurement used, i.e. it is a mere number.

The "Normal" Distribution

One type of symmetrical frequency distribution is known as the "*normal*" *distribution* (sometimes called the Gaussian distribution) and is of great theoretical and practical importance. Many measurable characteristics, e.g. stature, show this distribution, but its greatest importance lies in the field of "sampling" which will be discussed later. The essential characteristics of the "normal" frequency distribution are :—

(i) the mean, median and mode all coincide.
(ii) the curve is symmetrical about the mean.

(iii) the proportion of observations which will lie in the interval between the mean and the mean plus (or minus) any multiple of the standard deviation can be calculated.

e.g. the proportion of observations which lie within 1 S.D. from the mean, i.e. within the range $m - \sigma$ to $m + \sigma$ (where m=mean)
=68.27 per cent

the proportion of observations which lie within 2 S.D.
=95.45 per cent

the proportion of observations which lie within 3 S.D.
=99.73 per cent

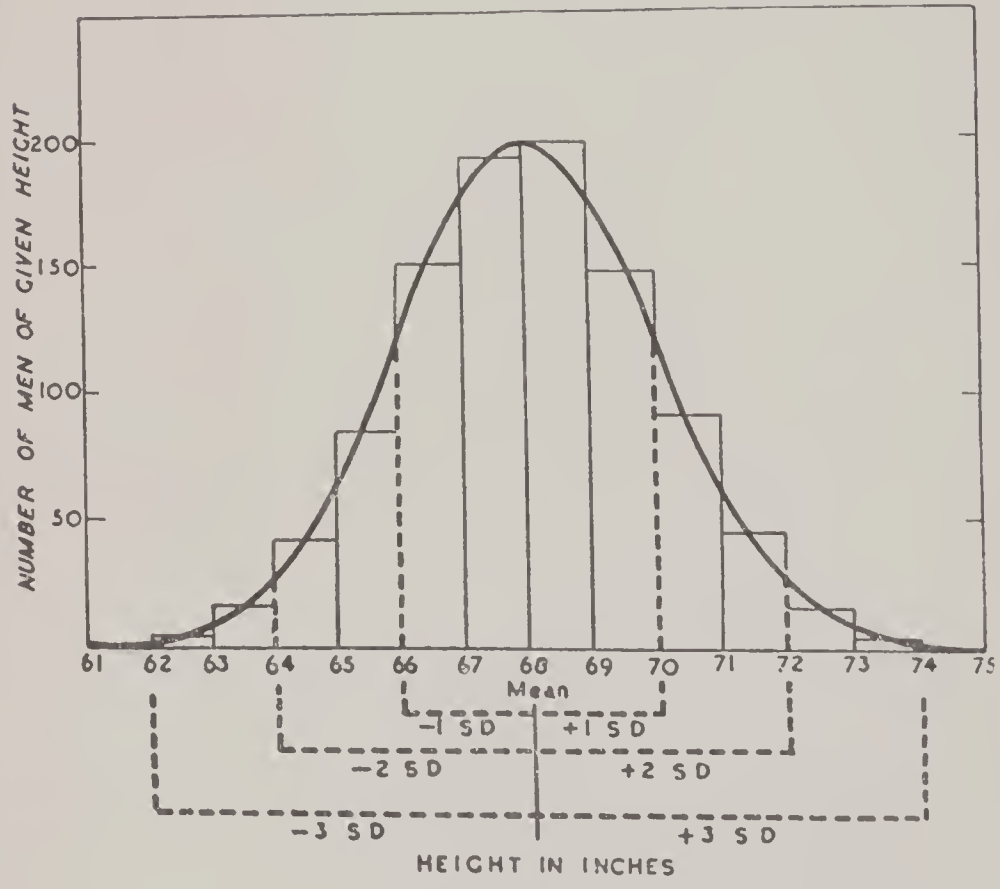

FIG. 46

NORMAL FREQUENCY DISTRIBUTION

Histogram based on hypothetical data of the stature of 1000 normal men ; a "Normal" Curve has been superimposed on the histogram and the distances of 1, 2 and 3 standard deviations from the mean are indicated.

(After Bradford Hill, 1948.)

Thus, with measurements that show a normal distribution, nearly 1 in 3 will differ from the mean value of the population by more than 1 S.D., 1 in 20 will differ from the mean by more than 2 S.D., and only 1 in 370 will differ from the mean by more than 3 S.D.

Problems of Sampling

In practical statistical problems it is nearly always the case that the observations utilized are only a sample from what statisticians term a "universe." The term "Universe" in this sense means "Universe of Discourse" and requires definition in all statistical investigations. Let us take an example. Suppose we were studying the effects of some new drug in the treatment of pneumonia, we should firstly define the criteria for diagnosis of this disease, and then, by methods to be discussed in this section, we should allocate a certain number of patients to "control" and "treated" groups. Now our particular study would necessarily be restricted both in time and in the number of patients treated, and, strictly speaking, our results would apply only to the recorded observations. But during the same period of time there would also be large numbers of other patients with pneumonia not included in the investigation, and the total number of these patients, plus our series, would be described as our universe of discourse, of which the cases we have studied would be only a sample. What we really want to know is (i) how far our results apply to the whole universe from which our patients were selected, and (ii) how frequently the observed difference between "control" and "treated" groups would occur as the result of random selection from this universe. This is the essence of the problem of sampling. A sample is a selected number of individuals each of which is a member of a particular universe. It is frequently, and rightly, assumed in ordinary life that samples give useful information about universes of various kinds, and it is also generally appreciated that the larger the sample becomes, the more accurately will it reflect conditions in the parent universe.

The problem of sampling is of great importance in experimental pharmacology and therapeutics, since it is impossible in practice to do more than a limited number of experiments to test a particular drug action. It is therefore necessary to discuss this question in some detail; practical illustrations will be given

later from recent therapeutic studies of the actions of streptomycin in pulmonary tuberculosis, and of anticoagulants in myocardial infarction.

Selection of the Sample

The first problem is the question of selection of individuals for the test since it is desired to make the sample as representative as possible of the universe as a whole. Now there are two main types of sampling: (*a*) *random*, (*b*) *purposive*. In random sampling each member of the universe has the same chance of being chosen, whereas in purposive sampling individuals are selected for some particular characteristic, e.g. sex or age group. It is clearly possible to combine the two procedures, e.g. after initial segregation of individuals into their appropriate sex and age groups, subsequent studies of the individuals allocated to each group could use the techniques of random sampling. In any case the aim is to eliminate as far as possible the errors which arise from conscious or unconscious human bias.

In therapeutic trials where it is desired to test the effect of a drug on the course of some disease, the most satisfactory procedure is to divide the patients approximately into two numerically equal groups, one of which receives the drug while the other group serves as a control. To elicit the greatest amount of information from the trial the two groups of patients should be the same in all relevant respects, apart from the administration of the drug. It is thus clear that great care must be taken to avoid bias in the allocation of patients to the two groups, and various methods have been adopted for this purpose.

(1) Alternate patients, regardless of their age, sex, or severity of illness, are allocated to the "treated" and "control" groups. Purposive selection into sex and age groups may precede such random allocation. A similar procedure is that in which patients admitted to hospital on odd days of the month are allocated to the treated group and those admitted on even days serve as controls.

(2) The sample of patients may be made more truly random by the use of special procedures, such as selection by means of random sampling numbers. Bradford Hill in the study on the effects of streptomycin in pulmonary tuberculosis to be described later, used such a technique so that for each sex, at each hospital

centre where the investigation was performed, a set of sealed, numbered envelopes contained randomly selected cards which assigned patients to the treated or control series. To avoid any psychological bias none of the investigators were told anything of the details of the method of selection, and the patients were not told that they were to receive any special treatment, or to act as controls. The only fact which might have aroused their suspicions was their more speedy admission to hospital!

Variability of Means of Samples

The accuracy of a value calculated from a sample depends on (i) the size of the sample, and (ii) the variability of the characteristic within the universe from which the sample is taken. Factor (i) is of course known, but (ii) is very rarely known, hence it is necessary to consider how it may be estimated, so that we can get some idea as to how representative our sample is of the universe as a whole.

If repeated samples of the same size are taken from a given universe the means, standard deviations and proportions will vary from sample to sample according to the laws of chance. It can, however, be proved that the means (and other statistics) of the samples will show a "normal" distribution about their true value in the universe. Thus, as in the case of the frequency distribution of a series of observations (p. 380), we can estimate the probability that the mean of a given sample will differ from the true mean by more than once, twice or three times the standard deviation. However it must be noted that the standard deviation of the mean of the universe is not known, since usually only one sample is taken, but it can be further shown that the standard deviation of the means of samples = the standard deviation of the individuals sampled in the population/the square root of the number of individuals in the sample. This value is termed the Standard Error (S.E.) of the mean and may be written thus :—

$$\text{S.E.} = \frac{\sigma}{\sqrt{n}}$$

This enables one to estimate how far the mean of a sample differs from the true mean of the universe, but it is important to realize that this relation is only applicable where n is large, and should be used with caution when n is less than 100.

Proportions and Percentage Statistics

Another statistical value of great practical importance is the proportion or percentage. It can be shown that the S.E. of a percentage is $\sqrt{\frac{p \times q}{n}}$ where p is the percentage of individuals belonging to one category (e.g. alive), q is the percentage belonging to the other category (e.g. dead), and n is the number of individuals in the sample.

Now the commonest problem in pharmacotherapy is to assess the effectiveness of a drug by comparing the results in a treated group of patients with those in some control group. If the control group is very large, as for example where it consists of many thousands of patients previously observed, the values of the mean and S.D. of this group may be regarded as the values for the universe, and it is then merely necessary to determine the S.E. of the mean, or the proportion, of the treated group. If these latter values differ markedly from those of the universe the probability of such a difference occurring by chance is very small, and it is very likely that the difference is due to the effect of treatment.

In most cases, however, the values for the universe are not known. For many reasons it is usually best to make simultaneous observations on treated and control patients, so the problem is how to test the significance of the observed differences between the two groups. There are two measures of great importance in this connection.

(1) *The Standard Error of the Difference between Two Means*

We have seen that the means and S.D. of samples of a given size taken from the same population are "normally" distributed about the true values for the whole population. It can be shown that the differences between the means of any two large samples from the same population are also "normally" distributed.

The S.E. of the difference between two means can be calculated in two ways which give closely similar results.

$$(a) \quad \text{S.E.} = \sqrt{\frac{\sigma^2}{n_1} + \frac{\sigma^2}{n_2}}$$

where σ = the standard deviation of all the observations in the two samples combined, and n_1 and n_2 = the numbers in the two samples.

$$(b) \quad \text{S.E.} = \sqrt{\frac{{\sigma_1}^2}{n_1} + \frac{{\sigma_2}^2}{n_2}}$$

where σ_1 and σ_2 = the standard deviations of the observations in the two samples, and n_1 and n_2 = the numbers of observations in the two samples.

(2) *The Standard Error of the Difference between Two Proportions*

This may also be calculated in two ways :—

$$(a) \quad \text{S.E.} = \sqrt{\frac{p \times q}{n_1} + \frac{p \times q}{n_2}}$$

where p and q are the percentages in both samples combined which have, and do not have, the characteristic, and n_1 and n_2 = the numbers of observations in the two samples.

$$(b) \quad \text{S.E.} = \sqrt{\frac{p_1 \times q_1}{n_1} + \frac{p_2 \times q_2}{n_2}}$$

where p_1 and q_1 are the percentages in one sample, p_2 and q_2 the percentages in the other, and n_1 and n_2 the numbers of observations in the two samples.

Significance of Differences

A very important question is the significance of differences between two means or proportions. All that is revealed by these tests is the probability of such differences occurring by chance, on the assumption that the samples are drawn from the same universe. If the observed difference between two means or proportions equals twice the value of the S.E. of the difference, such a difference would occur by chance roughly once in 20 tests ; if the observed difference is $2\frac{1}{2}$ times the S.E. it would occur by chance once in 80 tests, and if it is 3 times the S.E. it would occur once in 370 tests. It is clear then that these tests provide no positive proof or disproof of any hypothesis ; they merely indicate the likelihood of the observed differences being due to the chances of random sampling, and it is permissible to select any level of significance

for any particular type of investigation. The commonest procedure is to regard as significant a difference which exceeds twice the S.E.; this is called the 5 per cent level of significance since such a difference would occur by chance less than once in 20 tests. For many purposes, as in much experimental pharmacological work, this level is regarded as satisfactory but it is important to understand the arbitrary nature of this estimate. For other purposes the 1 per cent (or even 0.1 per cent) level of significance is desirable so that the probability of the observed differences occurring by chance is 1 in 100 (or 1 in 1,000).

The χ^2 Test

We have considered tests of significance of differences between means and proportions, but there are many cases where it is desired to compare the characteristics of two or more groups. For the comparison of such distributions Karl Pearson introduced what is called the χ^2 test ("chi squared" test). It is possible with this test to study the significance of differences between say various batches of serum in the prevention of measles, or between various drugs on a given infection.

A very useful application of the χ^2 test is to what is called the "Fourfold" Table.

TABLE 16

	No. of Individuals		
e.g.	Treated	Controls	Total
Survived . .	a	c	a+c
Died . .	b	d	b+d
Total . .	a+b	c+d	a+b+c+d

Suppose that a drug was being tested for its effect in some infection with a fairly high mortality rate (e.g. sulphonamides in pneumococcal pneumonia), the effectiveness of the drug could be assessed in terms of survival or death and the numbers in each group could be entered in the table as indicated above.

The value of χ^2 in this case can be calculated from the formula

$$\chi^2 = \frac{(ad-bc)^2(a+b+c+d)}{(a+b)(c+d)(a+c)(b+d)}$$

A table of values of χ^2 (e.g. in Fisher's Statistical Methods for Research Workers) is then consulted and the value of Probability (P) is read off against n (the no. of degrees of freedom) which for this 2×2-fold table=1. This gives the probability of the difference between the treated and control patients being due to sampling chances.

The Number of Observations Required for Significant Results

A question that naturally arises in therapeutic tests is how many patients should be included in each group to give significant results? This question cannot be answered in advance since it depends on the differences revealed in the course of the investigation. To quote an example given by Bradford Hill (*loc. cit.* p. 238), suppose that in a certain disease the fatality rate was 40 per cent in the control group and 20 per cent in the treated group, the question is to find the smallest number of patients which must be present in each group to make this difference statistically significant. If the difference between these proportions, i.e. 20 per cent, is equal to twice the standard error of the difference between the two proportions (which takes into account the numbers involved) it means that such a difference would occur by chance once in 20 trials (P=0.05). If this be taken as the level of significance it can be shown that 42 patients would have to be included in each group. If the fatality rates had been 40 per cent and 30 per cent respectively the number of patients in each group would have to be 182 to reach this level of significance, and if the fatality rates had been 4 per cent and 2 per cent the number required would be 600 in each group.

It will be noted that these examples assume the percentage differences to be established, but in practice these figures will not be known until some trials have been made, so it is clear that no accurate forecast can be made of the numbers required to establish a significant difference between the two groups.

Methods for Dealing with Small Samples

It has so far been assumed that the samples studied have been large, i.e. that they consisted of at least 50–100 observations. Where smaller numbers are concerned the measurement of the standard deviation is more accurately represented as

$$\text{S.D.} = \sqrt{\frac{\Sigma d^2}{n-1}} \text{ instead of } \sqrt{\frac{\Sigma d^2}{n}}$$

Indeed, the former is the correct representation for all cases, but when large numbers of observations have been collected no significant error results from the use of n instead of n−1.

When it is desired to estimate the significance of differences between means of small samples, what has been called the *t*-test, introduced by a Civil Service statistician who published his work under the nom-de-plume "Student," may be employed. An extension of "Student's" *t*-test to the comparison of two means has been described by R. A. Fisher and for details of its application the reader must be referred to this author's Statistical Methods for Research Workers. It should here be emphasized that with very small samples, e.g. four or five observations, statistically significant results cannot be obtained.

Examples of the Application of Statistical Methods in Therapeutic Trials

Streptomycin in Pulmonary Tuberculosis

A recent investigation on the short-term effects of streptomycin in the treatment of pulmonary tuberculosis will now be described to illustrate many of the factors which arise in the performance of a proper therapeutic trial. The inquiry was planned and directed by a special committee on behalf of the Medical Research Council and the results have been reported in the *British Medical Journal* (1948) (see also p. 160). At the commencement of this work in 1946 the supply of streptomycin was small, and it was felt that in a disease with such a variable and unpredictable natural course as pulmonary tuberculosis, the greatest care should be taken to use the available quantities to the best advantage. The first problem was the choice of the most suitable type of case, and for several reasons the patients were selected according to the following criteria: "acute progressive bilateral pulmonary

tuberculosis of presumably recent origin, bacteriologically proved, unsuitable for collapse therapy, age group 15–30." The selection of this type of disease fully justified the allocation of a number of patients to a parallel control series which was treated by bed-rest alone. 107 patients were studied in the trial, 55 receiving streptomycin and 52 serving as controls.

The allocation of the patients to the "streptomycin+bed-rest" group (S), or to the "bed-rest alone" group (C) was decided by a procedure based on random sampling numbers. Patients were not

TABLE 17

CONDITION ON ADMISSION								
GENERAL CONDITION	S GROUP	C GROUP	MAX. EVENING TEMP. IN FIRST WEEK	S GROUP	C GROUP	SEDIMENTATION RATE	S GROUP	C GROUP
GOOD	8	8	98—98·9°F (36·7—37·15°C.)	3	4	0–10	0	0
FAIR	17	20	99—99·9°F (37·2—37·75°C.)	13	12	11–20	3	2
POOR	30	24	100—100·9°F (37·8—38·25°C.)	15	17	21–50	16	20
			101°F (38·3°C.)+	24	19	51+	36	29
TOTAL	55	52	TOTAL	55	52	TOTAL	55	51

General condition, maximum evening temperature and blood sedimentation rate of patients with pulmonary tuberculosis before administration of streptomycin. S = Streptomycin treated group. C = Controls.

(Data from Medical Research Council Report, 1948.)

told before admission that they were to be given special treatment, and C patients did not know that they were acting as controls. After thorough initial clinical, radiological and pathological examinations each patient's condition was re-assessed at monthly intervals for six months. Particular care was taken in the interpretation of the X-ray films, which were all viewed independently by two radiologists and a clinician who did not know whether the films belonged to C or S cases.

Each patient was kept under observation for at least one week before the treatment or control period started. Table 17 shows

that most of the patients were acutely ill, and the data also show that random distribution had equalized the groups, with a slightly greater number of severe cases in the S group.

Streptomycin was given to S patients by intramuscular injections, the total daily dosage being 2 G., given in 4×0.5 G. doses 6 hourly. The duration of treatment in most of the patients was four months.

Results at the End of Six Months

Mortality Rate. 4 of the 55 patients (7 per cent) and 14 of the 52 C patients (27 per cent) died before the end of six months. The significance of this difference may be tested by determining the standard error of the difference between the two proportions. This is calculated from the formula

$$\sqrt{\frac{p_1 \times q_1}{n_1} + \frac{p_2 \times q_2}{n_2}}$$

p_1 and p_2=percentage of those dying, and q_1 and q_2=percentage of those surviving in each group, and n_1 and n_2 are the numbers in each group. Thus the standard error (S.E.) of the difference between the proportion in the S group and the proportion in the C group is

$$\sqrt{\frac{7 \times 93}{55} + \frac{27 \times 73}{52}} = 7.1\%$$

The observed difference between S and C groups is $27-7=20$ per cent which is nearly 3 times the S.E. The probability of this difference occurring by chance is less than 0.01 (1 in 100); other things being equal, and great care was taken to make them so, this suggests that streptomycin had a significant influence on the mortality rate at the end of six months.

An addendum to the report showed that at the end of one year 12 of 55 S patients (22 per cent) and 24 of 52 C patients (46 per cent) had died. Here the S.E. of the difference of the proportions is

$$\sqrt{\frac{22 \times 78}{55} + \frac{46 \times 54}{52}} = 8.9\%$$

The observed difference is 24 per cent which is over $2\frac{1}{2}$ times the S.E. of the difference between these proportions; the probability of this difference occurring by chance is less than 1 in 80.

Such differences in mortality rate are the simplest measures of effectiveness of the streptomycin treatment, but it is obviously desirable to take account of other factors in making a final assessment.

Radiological Appearances. At the end of six months the radiological assessment was "considerable improvement" in 28 of 55 S patients (51 per cent) and in 4 of 52 C patients (8 per cent). The probability of such a difference occurring by chance is less than 1 in 10 million.

It was also shown that the greater radiological improvement seen in the S cases was mainly in patients who were clinically acutely ill on admission. Thus among patients having initial evening temperatures of 101°F. or over, 13 of 24 S patients and 2 of 19 C patients showed radiological improvement, clearly a significant difference.*

Other Factors. Changes in general condition, temperature, body weight, sedimentation rate and bacillary content of the sputum were considered but are more difficult to evaluate numerically. The toxicity of streptomycin, and the development of drug resistance, were of course also studied but will not be discussed further here as they have been dealt with in Chap. 7.

This investigation has been discussed in detail (though by no means exhaustively) to illustrate how a therapeutic experiment can be designed and carried out. It will be noted that the cases were of only one type of pulmonary tuberculosis and the results here recorded might not apply to other types of the disease. Moreover a fixed daily dose (2 G.) of streptomycin was given. It is clear then that further questions remain to be answered, but it should at least be salutary to those who regard the therapeutic assessment of a drug as a simple problem, to realize how much work is required for the design, performance and interpretation of such an experiment.

Anticoagulant Therapy of Coronary Thrombosis with Myocardial Infarction

Another illustration of a controlled therapeutic test is provided by some recent American work reported by Wright *et al.* (1948).

*It will be noticed that the numbers of individuals in this case are small, and the significance of the differences would have to be assessed by the *t*-test previously mentioned (p. 389).

The problem was to assess the value of anticoagulant therapy in coronary thrombosis with myocardial infarction; the possible modes of benefit have been considered in Chap. 14. A special committee was appointed in 1946 by the Board of Directors of the American Heart Association to consider the question, and it was arranged that patients should be studied in 16 hospitals in different parts of the U.S.A. The results reported relate to 800 cases.

The principles of administration of the anticoagulants were briefly outlined. Heparin, if used, was given only during the first 48 hours of treatment, and generally dicoumarol alone was the basic anticoagulant throughout this work. The dosage of the latter was regulated to keep the prothrombin time between 30 and 50 sec. (normal = 15–17 sec.; 30–40 sec. = 10–20 per cent of normal prothrombin level). Dicoumarol therapy was maintained for at least 30 days, and preferably for 30 days after the last thromboembolic episode. The method of allocation of patients to the *Control* and *Treated* groups was simple, viz. those admitted on *even* days of the month served as controls, and those admitted on *odd* days were given anticoagulant therapy. The following table shows how this method of selection caused a very satisfactory distribution of the patients according to age, sex, etc., between the two groups :—

TABLE 18

	Controls	Treated
Number	368	432
Average Age	60 years	59 years
% males	77%	76%
One or more previous infarctions	24%	22%
Illness "severe" at onset	23%	30%
Anticoagulants	In 12% after complications	81% Dicoumarol alone 14% Dicoumarol + Heparin 5% Anticoagulants not given (owing to contraindications in 3%, and errors in 2%).

It will be noted from the table that 12 per cent of controls were later given anticoagulants ; it is also interesting to find that 3 per cent of the patients allocated to the treated group were considered unsuitable for anticoagulant therapy owing to renal or hepatic disease, or hæmorrhagic states.

The effects of anticoagulant therapy were assessed in a number of ways. The simplest numerical expression of the difference between the two groups is provided by the respective mortality rates—24 per cent in the *controls*, 14.9 per cent in the *treated* group. By calculating the standard error of the difference between these proportions it is shown that such a difference would occur by chance once in 900 tests.

The difference between treated and untreated cases could also be tested by calculating χ^2 from the "Fourfold" Table as follows :—

TABLE 19

	Number of Individuals		Total
	Treated	Not Treated	
No. survived . .	367 (a)	280 (c)	647 (a+c)
No. died . .	65 (b)	88 (d)	153 (b+d)
	432 (a+b)	368 (c+d)	

$$\chi^2=\frac{(ad-bc)^2(a+b+c+d)}{(a+b)(c+d)(a+c)(b+d)}=\frac{(32296-18200)^2(800)}{(432\times368\times647\times153)}=10.1$$

To find the value of P the table of χ^2 in Statistical Methods for Research Workers by Fisher (1946) is consulted. The value of P when $\chi^2=10.1$ and n (degrees of freedom)=1, is 0.0016. This means that the likelihood of such a difference arising by chance is about 1 in 630. These results show how even small percentage differences may be significant when large numbers of individuals occur in each group.

Another important observation reveals the benefits of anticoagulant therapy as follows :—

	Controls	Treated
Thromboembolic Complications before Death	10%	3%

This difference would occur by chance less than 1 in 10,000 times. Other data such as the death rates during different weeks of the illness, death rates by age groups, and types and locations of thrombo-embolic complications all showed differences between the control and treated groups, whose significance varied in such a way as to suggest that the effects of anticoagulant therapy were most marked during the first four weeks of the illness, and that the benefits were much greater in patients over, than in those under, 60 years of age.

The frequency of complications must of course be set against the benefits of this form of treatment. Hæmorrhage of various kinds is the only serious hazard, and was observed in 6 per cent of the control series and 12.4 per cent of the treated cases. Bleeding attributable to the anticoagulants occurred in 7 per cent of all treated patients. Of these 30 cases, 15 were classified as mild, 14 as moderate, and only one as severe.

The figures given in this report support the interpretation that anticoagulants improve the outlook for patients with coronary occlusion and myocardial infarction, and that, with proper laboratory control of the plasma prothrombin level (at 10–20 per cent of the normal), the occurrence of hæmorrhage rarely necessitates cessation of treatment.

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CHAPTER 18

THE CONTROL OF THE BODY FUNCTIONS BY CHEMICAL SUBSTANCES

One of the most important developments in modern pharmacology is the increasing realization that a number of processes which occur in the body, under normal and abnormal conditions, are under the control of chemical substances which, one by one, are being identified. It has long been known that the changes which occur in a single cell, obscure as they are, must be essentially of a physico-chemical nature. But the mechanisms by which the activities of cells are correlated and co-ordinated in a multicellular organism are only gradually being elucidated. The discovery of hormones marked a vital stage in this elucidation. It was then, for a time, believed that the hormonic mechanism of control, superimposed on and additional to that of the nervous system, involved the production of chemical substances, while the activity of the nervous system, central and peripheral, was dependent on changes which were essentially physical in nature. But the work and speculations of Elliot, Loewi and Dale presently showed how important a part is played by chemical substances in the transmission of the nervous impulse from the nerve to the affected organ, muscle or gland, and in the ganglia. And their pupils are now investigating to what extent these substances are also concerned in the transmission of the impulse in the central nervous system.

Another aspect of this same problem was opened up when it was realized that substances present in the food were also closely concerned in maintaining the normal activities of the body. It is difficult to believe, now that several dozen vitamins are already known, that it is less than 40 years ago that Hopkins made his original investigations on the effect of "accessory food factors" on the growth of rats. What makes these substances all the more interesting is the fact that some of them can be produced in the body of some animals, and that there is, in fact, no fundamental demarcation between vitamins and hormones.

Chemical substances too, are responsible for the processes of differentiation of organs which occur during development. These substances have been called "organizers" and their chemical nature requires much further investigation. Again, it is significant that the differentiation of certain sex organs is under the influence of the hormones which are concerned in the control of sexual activity in the adult, so that the difference between hormones and organisers is by no means sharp and clear cut.

The processes so far discussed are concerned with growth, differentiation and functional activity of the organism. Recently, however, it has been shown that chemical substances not only control these changes in the growing and formed organism, but that they may even be responsible for the formation of new types of organisms. Muller first showed that mutations, which are probably responsible, together with natural selection, for the development of new species, can be elicited by the action of X-rays and other forms of radiation. There has indeed been speculation as to whether radiant energy may not be responsible for most of the mutations which have occurred in the course of evolution. But in 1941 it was found that certain chemical substances can produce mutations, and mutagens, as these substances were called, are now being actively investigated, partly in the hope that specific effects may be discovered, and the process of mutation thus be selectively influenced.

Lastly, it must be realized that chemical substances produced in the body or derived from external sources not only can control and co-ordinate the activity of the cells which make up a multicellular organism, but that they can also create a certain degree of dissociation, which makes part of the organism independent of the rest, frequently leading to its death. Such effects are produced by carcinogenic substances which can transform normal into malignant cells. The carcinogens can certainly be introduced from outside, and may be produced in the organism as a result of abnormal metabolic processes, perhaps involving steroids. It is not really known how these carcinogenic substances produce their effect, but it is an interesting speculation that they do so by inducing mutations in somatic cells which then acquire a measure of independence from their environment. If so, mutagens and carcinogens would be similar substances acting at different stages of the life cycle, the mutagens affecting the sex cells and the

carcinogens acting on somatic cells, usually of the fully developed organism.

The substances which reach the circulating fluids and affect the activities of the cell can be described under the following headings. Some of the definitions have been taken from the discussion by Houssay (1946) :—

(1) Hormones, which are specific chemical substances produced by an organ or tissue and which, after being discharged into the circulating fluids, may reach all parts of the organism and in small amounts markedly influence the functioning of other organs or systems without themselves contributing important quantities of matter or energy.

(2) Chemical mediators of nerve action, liberated by nerve endings in close vicinity of effector organs or nerve cells and exerting a localized action. It is possible that similar substances are also involved in the transmission of impulses in the central nervous system.

(3) Vitamins, which are organic chemical regulators contained in the food. The nutritive substances in the food are of course also essential, in the final analysis, for the activities of the organism.

(4) Excretory products which nevertheless also perform important regulatory functions, e.g. carbon dioxide.

(5) Organisers which are embryonic substances of regional origin and govern the differentiation of a determined organ, even if transferred to another zone of the body, or cultivated *in vitro*.

(6) Carcinogens capable of changing normal cells into malignant ones.

(7) Mutagens, which act on the nucleus and change its inheritable material.

(8) Drugs introduced from the outside which produce pharmacological effects in the body.

Modern pharmacology is vitally interested in the mechanisms by which drugs, defined as chemical agents foreign to the body, interact with systems of control present within the body. It is of course still true that in many instances, e.g. the action of morphine, the mechanisms by which drugs produce their effects are unknown, but in other fields some progress has been made. For example, the pharmacological actions of anticholinesterase drugs are almost, if not entirely, due to their power of inhibiting the enzyme, cholinesterase, and the many different actions of vitamin

analogues may be aseribed to their antagonism towards the vitamins with which they are chemieally related. It would perhaps appear that the most fruitful field of the future will be a study of the various ways in which drugs can modify the aetivities of eonstituents of the cell, especially cellular enzymes, and inereasing knowledge of these and other types of interaction may help to fulfil the hope that ultimately the effects of all drugs will be expressed in such terms, thus leading not only to their more rational use, but also to more complete control of bodily processes by means of drugs.

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